8-2019

Prebiotic Carbohydrate Profiles of Lentil, Chickpea, and Common Bean

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PREBIOTIC CARBOHYDRATE PROFILES OF LENTIL, CHICKPEA, AND COMMON BEAN

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Plant and Environmental Sciences

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by
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August 2019

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ABSTRACT

Pulses such as lentil (*Lens culinaris* Medikus), common bean (*Phaseolus vulgaris* L.), and chickpea (*Cicer arietinum* L.) are a rich source of protein, prebiotic carbohydrates, and micronutrients. Prebiotic carbohydrates are utilized by beneficial gut microorganisms and produce short chain fatty acids which is associated with increasing mineral absorption and reducing obesity risk. The objectives of these studies were to 1) identify and quantify prebiotic carbohydrate profiles [simple sugars, sugar alcohols (SA), raffinose family oligosaccharides (RFO), fructooligosaccharides (FOS), resistant starch (RS), cellulose, hemicellulose, and amylose)] in different market classes of lentil, common bean, and chickpea, 2) determine the changes of SA, RFO, FOS, RS, and amylose concentration in common bean and chickpea market classes in response to cooking, cooling, and reheating, and 3) determine the changes of SA, RFO, FOS, RS, and amylose concentration in different market classes of lentil, common bean, chickpea in response to four cooking temperature ranging from 90 to 120 ºC.

The first study results indicated that a 100 g of lentil, common bean, and chickpea had 12, 15, and 12 g of prebiotic carbohydrates respectively. Prebiotic carbohydrate concentrations within the pulse market classes were significantly different. The second study results showed that a 100 g of cooked common bean and chickpea provide 7–9 and 8–10 g of prebiotic carbohydrates respectively. Cooling and reheating reduced SA and RFO but increased FOS, RS, and amylose concentrations regardless of the pulse market classes. The third study results showed that increasing cooking temperature from 90 ºC to 120 ºC, increased SA, RFO, FOS, and amylose concentration but reduced RS

concentration in pulse market classes. Overall, total prebiotic carbohydrates concentration was increased from 7 to 8 g/100 in lentil, 4 to 7 g/100 g in common bean, and 7 to 8 g/100 g in chickpea with increasing processing temperature.

In conclusion, prebiotic carbohydrate profiles are different in pulse market classes and it is possible to breed relevant pulse market classes with higher prebiotic carbohydrates. Further, processing methods change prebiotic carbohydrates concentration and therefore change the nutritional quality of pulses. Increasing cooking temperature up to 120 ºC increase prebiotic carbohydrates concentration in pulses. Thus, manipulation of processing conditions can be used to develop prebiotic carbohydrates rich pulse foods.

Keywords: pulses, prebiotic carbohydrates, thermal processing, shelf-stable foods

DEDICATION

This dissertation is dedicated to my loving wife, parents, brother, and friends for their early inspiration, coaching, and enthusiasm. None of this would have happened without them.

ACKNOWLEDGMENTS

I have been plentifully blessed during my degree program and research. Most of all, I thank my wife, parents, brother, and friends who have given me better life and shaped me into who I am. I offer my sincere thanks to my advisor, Dr. Dil Thavarajah and members of the graduate committee, Dr. Susan Duckett, Dr. William Whiteside, Dr. Pushparajah Thavarajah, and Dr. Elliot Jesch, who gave me tremendous support on this research. I would like to thank Dr. Susan Duckett's and Dr. William Whiteside's research team, Electron Microscope Facility, and Vegetable/Pulse Quality and Nutrition Laboratory research team at Clemson University. Also, I would like to thank Dhanuska Wijesinghe, Suranga Basnagala, Indika Pathirana, and Nathan Johnson for their support in my life. Finally, I would like to thank College of Agriculture, Forestry, and Life Sciences (CAFLS), Clemson University, the USDA National Institute of Food and Agriculture, the International Center for Dry Land Agriculture (ICARDA, Morocco), and the American Pulse Association for providing funds for this project.

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1. INTRODUCTION

Global populations are suffering from non-communicable diseases, overweight, and obesity. At present, 1.9 billion people in the world are overweight, 650 million are obese, 340 million children aged between 5–19 are overweight or obese, and 41 million children under the age of 5 are overweight or obese (WHO, 2018). Current overweight and obesity prevalence of adults in the USA is 33% and 38%, respectively (CDC, 2016). Unhealthy lifestyle including sedentary work and high intake of calorie dense foods increases obesity risk. Increase intake of vegetables, fruits, whole grains, and pulses rich in protein, minerals, vitamins, and prebiotic carbohydrates are recommended to combat obesity risk (CDC, 2015; WHO, 2003).

Prebiotic carbohydrates are defined as "*a substrate that is selectively utilized by host microorganisms conferring a health benefit*" (Gibson et al., 2017). Prebiotic carbohydrates fermented by beneficial gut microbiome and produce short chain fatty acids (acetate, butyrate, and propionate), regulate intestinal movement, prevent constipation, increase mineral absorption, and reduce obesity risk by regulating blood glucose and cholesterol levels (Kaur and Gupta, 2002; Manning and Gibson, 2004). Prebiotic carbohydrates include sugar alcohols (sorbitol, mannitol, xylitol, and galactinol), raffinose family oligosaccharides (raffinose, stachyose, and verbascose), fructooligosaccharides (kestose and nystose), resistant starch (RS), cellulose, hemicellulose, and pectin (Gibson et al., 2017). Jerusalem artichoke, green banana, onion, leeks, wheat bran, and pulses are rich source of prebiotic carbohydrates (Dwivedi et al., 2014; Raigond et al., 2015; Rubel et al., 2014).

Pulses including lentil (*Lens culinaris* Medikus), common bean (*Phaseolus vulgaris* L.), and chickpea (*Cicer arietinum* L.) are a rich source of protein and micronutrients, low in fat, and calorie (U.S. Pulse Quality Survey, 2017; USDA, 2019). A 100 g of serving pulses provide 22–28 g of protein, 2–3 g of micronutrients and only 0.1–5 g of fat and 300–350 kcal energy (U.S. Pulse Quality Survey, 2017; USDA, 2019). Pulses are a rich source of prebiotic carbohydrates (Johnson et al., 2013, 2015a). Lentil provides 1.2–1.5 g of SA, 5.5–6.1 g of RFO, 0-1 g of FOS, and 1.6–8.4 g of RS per 100 g of serving (Johnson et al., 2013). Further, chickpea and common bean provide 0.4–5.6 and 1.2–2.9/100 g of RFO (Gangola et al., 2016; Reddy et al., 1984), 0–1 and 0–0.07 g/100 g of FOS (Biesiekierski et al., 2011) and 2.9–4.5 and 2.4–4.4 g/100 g of RS, respectively (de Almeida Costa et al., 2006). Overall, pulses can provide 3–17 g of prebiotic carbohydrates per 100 g of serving (Biesiekierski et al., 2011; Gangola et al., 2016; Johnson et al., 2013; Reddy et al., 1984).

Prebiotic carbohydrate profiles change during food processing, cooking, and storage (de Almeida Costa et al., 2006; Johnson et al., 2015b; Siva et al., 2018). Cooking and microwave reheating of lentil reduce RFO concentration from 5.5–6.1 g/100 g to 4.3–4.9 g/100 g (Johnson et al., 2015b) and then cooling at 4 °C for 24 h increased RS concentration by twofold (Johnson et al., 2015b; Siva et al., 2018). These changes varied among lentil market classes, green lentil showed a higher reduction of RFO than whole red lentil (Johnson et al., 2015). These variations in the prebiotic carbohydrates in pulses are due to differences in chemical composition (i.e. amylose and amylopectin content) and physical properties of the seed (size and seed coat thickness) (Varatharajan et al.,

2011; Wang et al., 2003). Therefore, exploring prebiotic carbohydrates changes in different pulse market classes during processing will help to select prebiotic rich pulses to develop healthy diets.

Thermally processed shelf-stable foods are popular among consumers due to their conveniences. Canning reduced protein, dietary fiber, mineral concentration, and anti-nutrients such as phytic acid and tannins than the household cooked pulses (Margier et al., 2018). Due to low anti-nutrients in canned pulses, the bioavailability of nutrients is higher than the household cooked pulses (Margier et al., 2018). Considering prebiotic carbohydrates, canning beans at 118–122 ºC for 16 minutes reduced RFOs by 65% (Słupski and Gębczyński, 2014). Also, canning faba bean, kidney bean, and chickpea at 120 ºC for 15–20 minutes shows that RS levels were significantly reduced than its raw counterpart (Güzel and Sayar, 2012), but had more RS than ordinary boiled pulses (Güzel and Sayar, 2012). Thermal process has different effect depending on the type of pulse. Processing lentil at 159–161 ºC shows that raffinose level was significantly increased, verbascose level was significantly decreased, and stachyose had no difference than raw lentil (Morales et al., 2015), but similar processing condition increase both raffinose and stachyose in chickpea (Berrios et al., 2010). Therefore, the overall objective of this dissertation was to develop shelf-stable prebiotic rich pulse foods by characterizing prebiotic carbohydrates in pulses and optimizing the food processing conditions. Incorporating prebiotic rich pulses in the diet improve gut microbial compositions and lower the obesity risk.

2. CHAPTER ONE

VARIABILITY IN PREBIOTIC CARBOHYDRATES IN DIFFERENT MARKET CLASSES OF CHICKPEA, COMMON BEAN AND LENTIL COLLECTED FROM THE AMERICAN LOCAL MARKET

2.1. Hypotheses

H_0: Prebiotic carbohydrate profiles (simple sugars, SA, RFO, FOS, RS, cellulose, hemicellulose, and amylose) are not different within market classes of lentil, common bean, and chickpea.

H_1: Prebiotic carbohydrate profiles [simple sugars, sugar alcohols (SA), raffinose family oligosaccharides (RFO), fructooligosaccharides (FOS), resistant starch (RS), cellulose, hemicellulose, and amylose] are different within market classes of lentil, common bean, and chickpea.

2.2. Objective

Identify and quantify prebiotic carbohydrate profiles (simple sugars, SA, RFO, FOS, RS, cellulose, hemicellulose, and amylose) in two lentil market classes (red and green), seven common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto), and two chickpea market classes (desi and kabuli).

2.3. Abstract

Pulse crops such as lentil, common bean, and chickpea are rich in protein, low digestible carbohydrates, and range of micronutrients. The detailed information of low digestible carbohydrates also known as ‘prebiotic carbohydrate” profiles of commonly

consumed pulse market classes and their impact on human health are yet to be studied. The objective of this study was to determine the profiles of prebiotic carbohydrates in two commonly consumed lentil market classes, seven common bean market classes, and two chickpea market classes. After removing fat and protein, total carbohydrates averaged 51 g/100 g for lentil, 53 g/100g for common bean, and 54 g/100g for chickpea. Among the portion of total carbohydrates, lentil showed 12 g/100g of prebiotic carbohydrates (sum of sugar alcohols, raffinose family oligosaccharides, fructooligosaccharides, hemicellulose, cellulose, and resistant starch), 15 g/100 g in common bean, and 12 g/100 g in chickpea. Prebiotic carbohydrate concentrations within the market classes for each crop were significantly different ($P \leq 0.05$). In conclusion, these three pulses are rich in prebiotic carbohydrates, and considering the variation in these concentrations in the present materials, it is possible to breed appropriate market classes of pulses with high levels of prebiotic carbohydrates.

Keywords: Pulse crops, low digestible carbohydrates, prebiotic carbohydrates, resistant starch, amylose

2.4. Introduction

Carbohydrates are widely present in plants and animals and are used as an energy source to fulfill metabolic requirements (Trumbo et al., 2002). Carbohydrates are classified into three major groups, simple sugars, oligosaccharides, and polysaccharides or complex carbohydrates, based on their chemical structure. Complex carbohydrates have a degree of polymerization 10 or more than the simple and oligosaccharides. Prebiotic carbohydrates, a category of oligosaccharides and complex carbohydrates also

known as low digestible carbohydrates, are defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Manning and Gibson, 2004). Despite several modifications to the definition, a prebiotic carbohydrate is a specific colonic nutrient that acts as a biosynthetic precursor for human microbiota activity (Hutkins et al., 2016). Classification of a food as a prebiotic carbohydrate requires that the ingredient: (1) resists digestive processes in the upper part of the gastrointestinal tract, (2) is fermented by intestinal microbiota, and (3) selectively stimulates growth and activity of health-promoting bacteria (Manning and Gibson, 2004). Simple carbohydrates are comprised of one sugar unit (monosaccharides) or two sugar units (disaccharides) that are easily digestible, whereas oligosaccharides have 3 to 10 sugar units and complex carbohydrates feature more than ten sugar units (polysaccharides) (Cummings and Stephen, 2007).

Oligosaccharides and complex carbohydrates provide prebiotic health benefits by modulating healthy gut bacteria (Oku and Nakamura, 2003; Manning and Gibson, 2004). Whole grains are rich in prebiotic carbohydrates, but most food processing techniques remove prebiotic carbohydrates, especially in cereals, i.e., white bread and breakfast cereal, so consumption of such foods can lead to an increased risk of obesity and related non-communicable diseases (Hodge et al., 2004). Pulse crops, such as lentil (*Lens culinaris* Medikus.), common bean (*Phaseolus vulgaris* L.), and chickpea (*Cicer arietinum* L.) are consumed as whole foods and require minimal or no processing, and therefore contain higher amounts of prebiotic carbohydrates than processed cereals and

other grains (Bhatty, 1988; Guillon and Champ, 2002; Johnson et al., 2013). Diets rich in prebiotic carbohydrates change the gut microbial composition, lead to production of fatty acids (acetate, butyrate, and propionate), regulate intestinal movement, and prevent constipation (Manning and Gibson, 2004). Additionally, such diets tend to increase mineral absorption and reduce obesity risk by regulating blood glucose and cholesterol levels (Kaur and Gupta, 2002). However, the current daily intake of prebiotic carbohydrates in Western populations is less than 50% of the recommended daily allowance (RDA) (Van Loo et al., 1995), but can be increased by incorporating pulses in the diet.

The benefits of prebiotic carbohydrates are not limited to humans, but also extend to plant health by increasing stress tolerance to cold and drought. For example, leaf raffinose family oligosaccharides (RFOs) enhance drought (Bartels and Sunkar, 2005), chilling (Liu et al., 2007; Nishizawa et al., 2008), and freezing tolerance in plants (Pennycooke et al., 2003). Further, sugar alcohols (SAs; sorbitol and mannitol) increase tolerance to chilling (Chiang et al., 2005), drought (Pujni et al., 2007), and salinity (Tang et al., 2005; Zhifang and Loescher, 2003). RFOs and SAs act as osmolytes to maintain cell structure during drought and salt stress (Bartels and Sunkar, 2005; Pharr et al., 1995) and as antioxidants to neutralize the reactive oxygen species that cause cell damage (Keunen et al., 2013; Liu et al., 2007; Nishizawa et al., 2008). Further, SAs and RFOs act as signaling compounds for biotic stress caused by insects and pathogens (Kim et al., 2008; Valluru and Van den Ende, 2011).

Current annual lentil, common bean, and chickpea production around the world is approximately 6, 12, and 26 million tons, respectively (FAO, 2018). With climate change, future pulse crop production might be limited because of increased drought and temperatures. As such, developing climate resilient and nutritionally superior cultivars via plant breeding and selection is essential for future pulse crop improvement and global food security (Muehlbauer et al., 2006). A 100 g serving of lentil contains 1-2 g of SA, 5-6 g of RFO, 0-1 g of fructooligosaccharides (FOS), and 2-8 g resistant starch (RS) (Johnson et al., 2013). However, very limited information in terms of detailed profiles of prebiotic carbohydrates is available for other pulses, including chickpea and common bean. The objective of this study was to identify and quantify prebiotic carbohydrate profiles (simple sugars, SA, RFO, FOS, RS, cellulose, hemicellulose, amylose) in two lentil market classes (red and green), seven common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto), and two chickpea market classes (desi and kabuli).

2.5. Materials and Methods

2.5.1. Materials

Chemicals used for high performance anion exchange chromatography (HPAE) and enzymatic assays were purchased from Fisher Scientific (Asheville, NC, USA), Sigma-Aldrich (St. Louis, MO, USA), and VWR International (Satellite Blvd, Suwanee, GA, USA). Distilled and deionized water (ddH2O) with a resistance of ≥18.2 MΩ (NANO-pure Diamond, Barnstead, IA, USA) was used in these analyses.

2.5.2. Lentil, common bean and chickpea seeds

Approximately 4 kg of five commercially available lentil seed samples from two market classes (red and green) were collected from the Northern Pulse Growers Association, ND, USA. The red market class included whole seed (with seed coat), dehulled (whole seed without seed coat), and dehulled split (split seed without seed coat) and the green market class included whole seed and dehulled split **(Table 2.1)**. Samples (approximately 2 kg) of seven commercially available common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto) grown in the USA were obtained from local grocery stores, and two chickpea market classes (desi and kabuli) were obtained from a commercial pulse distributor (AGT Foods, Bismarck, ND, USA) **(Table 2.1)**. These different pulse seed sample were collected from regional pulse distributors and local market, therefore additional information on growing conditions, soil management, and variety information were not available.

Table 2. 1. Description of pulse market classes used in this experiment.

Type	Market class	Commercial form	1000 seed weight (g)
Lentil	Red	Whole (with seed coat)	29
		Dehulled dehulled	33
		Dehulled split	16
	Green	Whole (with seed coat)	46
		Dehulled split	45
Common bean	Small red	Whole (with seed coat)	315
	Cranberry	Whole (with seed coat)	569
	Great northern	Whole (with seed coat)	338
	Light red kidney	Whole (with seed coat)	593
	Black	Whole (with seed coat)	182
	Navy	Whole (with seed coat)	198
	Pinto	Whole (with seed coat)	344
Chickpea	Desi	Whole (with seed coat)	228
	Kabuli	Whole (with seed coat)	473

Samples were cleaned by hand, homogenized, subsampled, and ground to a 1-mm particle size using a cyclone mill (CT 193 Cyclotec Sample Mill, FOSS North America, MN, USA). The treatment design was a completely randomized design with five lentil types, seven common bean types, and two chickpea types (n=14) and three replicates (n=3), for a total of 42 (n=42).

2.5.3. Fat and protein removal

Ground seed samples were dried at 100-102 °C for 3 h. Fat was removed with hexane at 90 °C for 2 h in an ANKOM extractor (XT15, Macedon, NY, USA). Defatted samples were treated with 0.2% NaOH (1:6; w/v) in a water bath at 45 °C for 90 min to remove protein (Neethirajan et al., 2012; Sivapragasam et al., 2014). Samples were then blended for 2 min and centrifuged at 3000 x *g* (Fisher Scientific, Waltham, MA, USA) for 15 min. The supernatant was discarded, and the top layer was removed. Ten mL of ddH_2O were added, the solution was mixed and centrifuged, and the supernatant and top layer was removed. This process was repeated until the top yellow layer no longer visible. The suspension was re-suspended with 10 mL of ddH_2O and adjusted to a pH of ~7 with 50 mM HCl (Sivapragasam et al., 2014). Following centrifugation, samples were washed three times with ddH_2O and air dried at 60 °C overnight.

2.5.4. Low molecular weight carbohydrates (LMWC)

Ground seed samples (500 mg) were weighed into 15-mL polypropylene conical tubes. Ten mL of ddH_2O were then added to the tubes, which were incubated for 1 h at 80 °C as per Muir et al. (2009). Samples were centrifuged at 3000 x *g* for 10 min. An aliquot (1 mL) of the supernatant was diluted with 9 mL of ddH_2O, and the diluted supernatant

was filtered through a 13 mm × 0.45 μm nylon syringe filter (Fisher Scientific, Waltham, MA, USA) prior to HPAE analysis.

Low molecular weight carbohydrate concentrations (SA, RFO, and FOS) were measured using HPAE (Dionex, ICS-5000, Sunnyvale, CA, USA) according to a previously published method (Feinberg et al., 2009). SA, RFO, and FOS were determined by running the mobile phases (A: 100 mM sodium hydroxide/600 mM sodium acetate; B: 200 mM sodium hydroxide; C: ddH_2O) at a flow rate of 1 mL/min through a CarboPac PA1 column (250 × 4 mm; Dionex, CA, USA) connected to a CarboPac PA1 guard column (50 × 4 mm; Dionex, CA, USA). The total run time was 25 min. Detection was carried out using a pulsed amperometric detector (PAD; ICS-5000, Thermo Scientific, Waltham, MA, USA) with a working gold electrode and a silver-silver chloride reference electrode at 2.0 μA. Sugar alcohols (sorbitol and mannitol), RFO (raffinose, stachyose, and verbascose), and FOS (kestose and nystose) were identified and quantified using pure standards (>99%), and low molecular weight carbohydrate concentrations were detected within a linear range of 3 to 1000 μg/g with a minimum detection limit of 0.2 μg/g. A lab reference (CDC Redberry lentil) was used to ensure the accuracy and reproducibility of detection. The peak areas of the external reference, glucose (100 ppm), SA (3-1000 ppm), RFO (3-1000 ppm), and FOS (3-1000 ppm) were routinely analyzed for method consistency and detector sensitivity, with an error of less than 5% (Johnson et al., 2013). The concentration of LMWC in the samples (C_s) was calculated according to $C_s = (C_f \times V) / m$, where C_f is the filtrate concentration obtained from HPAE, V is the final diluted volume, and m is the mass of the sample (moisture corrected). Unidentified compound

concentrations were determined based on of those identified carbohydrate peak areas that were very closest to retention times.

2.5.5. Hemicellulose

Samples weighing 500 mg were loaded into 15-mL polypropylene conical tubes, which were incubated with 5 mL of 7% (w/w) HCl at 55 °C for 120 min followed by centrifugation at 3000 x *g* for 10 min (Thavarajah et al., 2016). Concentrations of arabinose and xylose were measured using the HPAE-PAD method described above. Hemicellulose concentration was reported as the summation of arabinose and xylose concentrations, and then multiplied by 0.9. Pectin concentration was not measured.

2.5.6. Cellulose

Cellulose was measured using enzymatic hydrolysis of cellulose (Lee et al., 2009). Cellulase enzyme (extracted from *Aspergillus niger*, 1 U of enzyme liberates 1.0 μmole of glucose at 37 °C for 1 h incubation) was purchased from Sigma-Aldrich, St. Louis, MO, USA. Samples (100 mg) were weighed into 15-mL polypropylene conical tubes. An aliquot (3.5 mL) of cellulase (34 U/mL in 50 mM citrate buffer, pH 4.7) was added and the mixture incubated in a water bath (Orbit shaker bath, Lab Line Instruments Inc., Melrose Park, ILL) with a rotary shaker (200 rpm) at 37 °C for 10 h (Lee et al., 2009). Tubes were then centrifuged at 3000 x *g* for 10 min and 1 mL of the supernatant then diluted with 19 mL of ddH_2O. The total glucose concentration resulting from cellulose hydrolyzation was measured using an enzymatic assay (Megazyme, 2012). Aliquots (0.1 mL) of diluted solution and glucose standard (1 mg/mL) were added separately to 10-mL round bottom glass tubes. Then, 3 mL of GOPOD reagent (12,000

U/L glucose oxidase, 650 U/L peroxidase, and 0.4 mM 4-aminoantipyrine, pH 7.4) were added to each tube, which were then incubated in a water bath at 50 °C for 20 min. The absorption of the samples was measured using a spectrophotometer (Genesys 20, Thermo Scientific, NC, USA) at 510 nm (the absorbance value of the glucose standard) to determine the concentration of glucose in the samples. The cellulose concentration was determined by multiplying the glucose concentration by 0.9 (the ratio of free glucose to anhydro-glucose that occurs in cellulose).

2.5.7. Resistant starch

RS concentrations were determined according to McCleary and Monaghan, (2002) and Megazyme, (2012). Ground samples (500 mg) were incubated with 4 mL of 100 mM sodium malate (pH 6) containing α-amylase (10 mg/mL) and amyloglucosidase (3 U/mL) for 16 h in a water bath (37 °C) with 200 strokes/min vertical shaking (Orbit shaker bath, Lab Line Instruments Inc., Melrose Park, IL, USA). After incubation, 4 mL of 95% ethanol were added, and the samples were then centrifuged at 1500 x *g* for 10 min at room temperature. The pellets were re-suspended with 6 mL of ethanol (50% v/v), centrifuged, and decanted. The resuspension and centrifugation process were done two times. Supernatants from the three centrifugations were pooled and brought to a volume of 100 mL with ddH_2O. The pellets were dissolved in 2 mL of potassium hydroxide (2 M) in an ice bath (~0 °C) while stirring with a magnetic stirrer for 20 min. The suspensions were diluted with 8 mL of sodium acetate buffer (1.2 M, pH 3.8), with 0.1 mL of 3300 U/mL amyloglucosidase then immediately added followed by incubation at 50 °C for 30 min. The suspension was then centrifuged at 1500 x *g* for 10 min at room

temperature. Aliquots (0.1 ml) of both the supernatant containing the RS fractions and the diluted washings containing the soluble starch (SS) fractions were transferred separately to 10-mL glass tubes. A reagent blank was prepared using 0.1 mL sodium acetate buffer (pH 4.5). An aliquot (3 mL) of GOPOD reagent was added to each tube, which were incubated in a water bath at 50 °C for 20 min. Absorption was measured using a spectrophotometer (Genesys 20, Thermo Scientific, NC, USA) at 510 nm. Starch fractions were calculated as follows:

$$RS = \frac{X \times (Abs_{sample})}{(Abs_{glucose} \times W_{sample})},$$

$$SS = \frac{Y \times (Abs_{sample})}{(Abs_{glucose} \times W_{sample}},$$

where Abs_{sample} and $Abs_{glucose}$ are the absorbance value of sample and glucose corrected against reagent blank, respectively; W_{sample} is the moisture corrected weight of sample; and X and Y are the dilutions factors for RS and SS, respectively. Regular corn starch (RS concentration 1.0±0.1% (w/w)) was used to verify the data, and batches were checked regularly to ensure an analytical error of less than 10%.

2.5.8. Amylose and amylopectin

Amylose levels were determined using an enzymatic assay (Gibson et al., 1997; Magazyme, 2016). Samples (20-25 mg) of defatted and deproteinated flour were transferred to 15-mL screw capped polypropylene conical tubes. An aliquot (1 mL) of dimethyl sulphoxide (DMSO; 99.5% v/v) was added to each tube, which were heated for 1 min in a boiling water bath. The tube contents were then vigorously mixed in a high-speed vortex and heated for 15 min in a boiling water bath. The tubes were cooled to

room temperature, and an aliquot (2 mL) of ethanol (95% v/v) added during continuous stirring. Then 4 mL of ethanol were added to the samples, which were allowed to stand for 15 min after thorough mixing. The tubes were centrifuged at 2000 x *g* for 5 min, and the supernatant discarded. Two mL of DMSO were added, and the samples heated for 15 min in a boiling water bath with occasional mixing. Immediately after their removal, 4 mL of concanavalin A (Con A) buffer (180 mM sodium acetate buffer, pH 6.4) were added to the samples, which were mixed thoroughly. The contents were diluted with Con A buffer to 25 mL (Solvent A).

Aliquots (1 mL) of diluted solvent A were transferred to 2-mL microfuge tubes to which 0.5 mL of lectin Con A solution (6 mg/mL) was added. The tubes were mixed gently by repeated inversion and incubated for 1 h at room temperature followed by centrifugation at 14,000 x *g* for 10 min. The supernatant (1 mL) was transferred to a 15-mL centrifuge tube and 3 mL of sodium acetate buffer (100 mM, pH 4.5) then added. The contents were mixed in a boiling water bath for 5 min and incubated at 40 °C for 5 min. Four mL of 100 mM sodium acetate buffer were added to 0.5 mL of solvent A. An aliquot (0.1 mL) of amyloglucosidase (333 U/ml)/ α-amylase enzyme (67 U/mL) was added to the tubes containing either diluted solvent A or con A supernatant, which were then incubated at 40 °C for 10 min followed by centrifugation at 2000 x *g* for 5 min. An aliquot (4 mL) of GOPOD reagent was added to 1 mL of supernatant and incubated at 40 °C for 20 min. Absorbance was measured at 510 nm in a spectrophotometer, with the percent amylose and amylopectin measured as follows:

$$\text{Amylose (\%)} = \frac{\text{Abs}_{\text{(Con A supernatant)}}}{\text{Abs}_{\text{(Total starch aliquot)}}} \times \frac{6.15}{9.2} \times 100 \text{ ,}$$

Amylopectin (%)=100%-Amylose (%) ,

where 6.15 and 9.2 are dilution factors for the Con A and total starch extracts, respectively.

2.5.9. Statistical analysis

Lentil, common bean, and chickpea market classes and replicates were considered as random factors and included as class variables. Analysis of variance (ANOVA) was performed using the General Linear Model procedure (PROC GLM) of SAS version 9.4 (SAS, 2016) and Fisher's protected least significant difference (LSD) at $P \leq 0.05$ was used to separate means.

2.6. Results

Total carbohydrate concentrations averaged 51g/100g in lentil, 53 g/100g in common bean, and 54 g/100g in chickpea, while total prebiotic carbohydrates averaged 12 g/100g in lentil, 15 g/100g in common bean, and 12 g/100 g in chickpea **(Table 2.2)**. Sugar alcohols and oligosaccharide concentrations were generally higher in lentil whereas hemicellulose, cellulose, resistant starch, amylose, and amylopectin were slightly higher in common bean and chickpea.

2.6.1. Lentil

Among simple sugars, sucrose was the most abundant (1.2-2.3 g/100 g) followed by glucose (21-61 mg/100 g), fructose (0.2-21.9 mg/100 g), mannose (1.2-7.9 mg/100 g), and rhamnose (0.5-1.0 mg/100 g) **(Table 2.3)**. For SAs, lentil contained higher concentrations of sorbitol (606-733 mg/100 g) than mannitol (9-31 mg/100 g) and xylitol

(14-31 mg/100 g) regardless of market class **(Table 2.4)**. Whole red had significantly ($P < 0.05$) higher levels of sorbitol than all other market classes, and whole green had significantly higher mannitol and xylitol concentrations. For RFO, stachyose concentrations (2.2-2.3 g/100 g) were higher than raffinose (403-646 mg/100 g) and verbascose (581-1769 mg/100 g) concentrations **(Table 2.5)**. Considering lentil FOS, concentrations of kestose were considerably higher than those for nystose. Arabinose concentrations were significantly higher in whole green compared to red split lentil **(Figure 2.1a)**. Among the market classes, red dehulled and red split had significantly higher xylose concentrations (1.91-1.94 g/100 g) than the other market classes. Whole red and whole green had significantly higher cellulose concentrations (611-640 mg/100 g) than the other market classes **(Figure 2.1a)**. Soluble starch concentrations ranged from 37 to 44 g/100 g with levels in red dehulled and dehulled green significantly higher than those in whole red and red split **(Figure 2.2a)**. No significant differences were observed for RS levels among market classes; however, amylose concentrations were significantly higher in red dehulled, whole green, and dehulled green than in whole red **(Figure 2.2a)**.

2.6.2. Common bean

Among simple sugars, sucrose was the most abundant (2.6-3.7 g/100 g) followed by glucose (35-62 mg/100 g), fructose (1.7-16.4 mg/100 g), mannose (1.5-11.2 mg/100 g), and rhamnose (0.1-0.7 mg/100 g) **(Table 2.3)**. Considering SAs, common beans had higher concentrations of mannitol (3-13 mg/100 g) than sorbitol (0.1-2.3 mg/100 g) and xylitol (1.9-8.6 mg/100 g) **(Table 2.4)**. Among market classes, light red kidney bean had significantly ($P < 0.05$) higher mannitol concentrations and black bean had higher

Table 2. 2. Prebiotic carbohydrate profiles of lentil, common bean, and chickpea.

Carbohydrates	Lentil	Common bean	Chickpea
Sugar alcohols (mg/100 g)	707±51	11±3	548±53
Simple sugars			
Monosaccharides (mg/100 g)	44±23	66±15	34±4
Disaccharides (g/100 g)	1.7±0.4	3.1±0.4	2.2±0.4
Oligosaccharides			
Raffinose family oligosaccharides (g/100 g)	4.1±0.5	3.0±0.3	2.1±0.2
Fructooligosaccharides (mg/100 g)	333±80	52±13	46±16
Polysaccharides			
Hemicellulose (g/100 g)	3.8±0.2	7.9±0.5	6.1±0.5
Cellulose (g/100 g)	0.5±0.2	1.6±0.9	1.1±0.3
Soluble starch (g/100 g)	40±3	41±3	42±4
Resistant starch (g/100 g)	2.1±0.3	2.4±0.4	3.1±0.1
Amylose (g/100 g)	17±2	19±2	19±2
Amylopectin (g/100 g)	25±2	24±2	26±2
Unidentified** (mg/100 g)	426±39	151±28	183±80
Total prebiotic carbohydrates (g/100 g)	12±1	15±1	12±2
Total identified carbohydrates (g/100 g)	51±2	53±2	54±7
RDA from a 100 g serving (%)	60±6	75±5	60±8

Data represent mean value ± standard deviation. Values are presented on a wet weight basis (10%). Recommendations for safe daily total prebiotic intake (20 g/day) reported by Douglas & Sanders, 2008. Unidentified compound concentrations were determined based on of those identified carbohydrate peak areas that were very closest to retention times.

Table 2. 3. Concentration of simple sugars of different lentil, common bean, and chickpea market classes.

Market class	Concentration (mg/100 g)				
	Mannose	Glucose	Fructose	Sucrose	Rhamnose
Lentil					
Whole red	1.5±0.7 *c*	60.5±7.7 *a*	21.9±2.6 *a*	1174±89 *e*	0.7±0.2 *b*
Red dehulled	5.6±0.3 *b*	24.6±1.3 *c*	0.5±0.1 *c*	2057±94 *b*	0.5±0.0 *b*
Red split	7.9±0.9 *a*	21.1±1.0 *c*	0.3±0.1 *c*	2288±76 *a*	0.7±0.2 *b*
Whole green	1.2±0.3 *c*	42.2±5.4 *b*	4.5±2.0 *b*	1665±25 *c*	1.0±0.0 *a*
Dehulled green	1.8±0.2 *c*	24.3±4.8 *c*	0.2±0.1 *c*	1376±140 *d*	0.5±0.0 *b*
Mean	3.6±2.8	34.6±16.0	5.5±8.8	1712±435	0.7±0.2
Common bean					
Small red	9.5±7.0 *a*	57.9±8.9 *ab*	12.6±6.6 *a*	3287±115 *b*	0.2±0.0 *c*
Cranberry	3.6±2.0 *cb*	54.6±6.9 *cb*	5.4±5.2 *cb*	3710±73 *a*	0.7±0.1 *a*
Great northern	10.5±1.0 *a*	46.5±2.9 *ed*	5.2±1.4 *cb*	3296±116 *b*	0.1±0.0 *c*
Light red kidney	7.9±2.6 *ab*	49.9±3.9 *cd*	12.6±8.6 *a*	3188±29 *b*	0.3±0.0 *b*
Black	1.5±0.1 *c*	62.1±4.1 *a*	16.4±0.9 *a*	2605±94 *c*	0.2±0.0 *c*
Navy	11.2±0.8 *a*	41.8±0.4 *ef*	1.7±0.7 *c*	2637±30 *c*	0.2±0.0 *c*
Pinto	1.7±0.6 *c*	34.7±2.4 *f*	10.0±0.9 *ab*	2660±113 *c*	0.1±0.0 *c*
Mean	6.6±4.7	49.6±10.0	9.1±6.2	3055±412	0.3±0.2
Chickpea					
Desi	0.8±0.2 *a*	29.6±6.4 *a*	2.2±0.2 *a*	1764±104 *b*	0.1±0.0 *a*
Kabuli	0.5±0.1 *b*	31.8±0.6 *a*	2.5±0.3 *a*	2541±69 *a*	0.1±0.0 *a*
Mean	0.6±0.2	31.7±4.2	2.4±0.3	2153±433	0.1±0.0

Data represent mean value ± standard deviation. Values are presented on a wet weight basis (10% moisture). Values within each market class followed by a different letter are significantly different at $P < 0.05$ (n=42).

Table 2. 4. Concentration of sugar alcohols (sorbitol, mannitol, and xylitol) of different lentil, common bean, and chickpea market classes.

Market class	Concentration (mg/100 g)		
	Sorbitol	Mannitol	Xylitol
Lentil			
Whole red	733±44 *a*	9±1 *d*	14±1 *d*
Red dehulled	606±24 *c*	21±1 *c*	24±1 *c*
Red split	649±23 *cb*	22±4 *c*	28±1 *b*
Whole green	631±7 *cb*	31±1 *a*	31±1 *a*
Dehulled green	690±61 *ab*	27±4 *b*	22±2 *c*
Mean	662±56	22±8	24±6
Common bean			
Small red	0.8±0.0 *c*	4.1±0.3 *c*	3.8±0.1 *c*
Cranberry	0.7±0.0 *c*	8.8±0.6 *b*	1.9±0.3 *e*
Great northern	0.2±0.0 *e*	3.7±0.3 *cd*	4.9±0.3 *b*
Light red kidney	0.1±0.1 *e*	12.7±0.3 *a*	3.1±0.1 *d*
Black	2.3±0.2 *a*	3.1± 0.1 *ed*	8.6±0.3 *a*
Navy	0.4±0.1 *d*	3.0± 0.4 *e*	3.5±0.4 *cd*
Pinto	1.2±0.2 *b*	3.2± 0.1 *ed*	3.7±0.4 *c*
Mean	0.8±0.7	5.5±3.6	4.2±2.0
Chickpea			
Desi	557±16 *a*	19±6 *a*	18±1 *a*
Kabuli	473±8 *b*	15±5 *a*	14±0 *b*
Mean	515±48	17±6	16±2

Data represent mean value ± standard deviation. Values are presented on a wet weight basis (10% moisture). Values within each market class followed by a different letter are significantly different at $P < 0.05$ (n=42).

Table 2. 5. Raffinose family oligosaccharides (raffinose, stachyose, and verbascose) and fructooligosaccharides (kestose and nystose) concentrations in different lentil, common bean, and chickpea market classes.

Market class	RFO (mg/100 g)			FOS (mg/100 g)	
	Raffinose	Stachyose	Verbascose	Kestose	Nystose
Lentil					
Whole red	492±119 *ab*	2294±35 *a*	581±51 *c*	191±16 *b*	0.01±0.00 *b*
Red dehulled	464±32 *ab*	2236±107 *a*	1435±74 *b*	349±20 *a*	0.01±0.00 *b*
Red split	646±144 *a*	2348±198 *a*	1769±43 *a*	391±25 *a*	0.01±0.00 *b*
Whole green	477±26 *ab*	2290±71 *a*	1653±68 *a*	382±2 *a*	0.01±0.00 *b*
Dehulled green	403±96 *b*	2292±66 *a*	1333±153 *b*	353±61 *a*	0.08±0.04 *a*
Mean	496±116	2292±100	1354±437	333±80	0.02±0.03
Common bean					
Small red	721±114 *a*	2492±62 *a*	128±31 *b*	45±4 *cb*	0.01±0.00 *a*
Cranberry	644±65 *ab*	2436±70 *ab*	187±16 *a*	56±4 *ab*	0.01±0.00 *a*
Great northern	626±47 *ab*	2315±8 *b*	157±21 *ab*	43±6 *cb*	0.01±0.00 *a*
Light red kidney	717±31 *a*	2093±30 *c*	181±16 *a*	69±4 *a*	0.01±0.00 *a*
Black	754±103 *a*	2404±130 *ab*	166±24 *ab*	68±14 *a*	0.01±0.00 *a*
Navy	642±31 *ab*	2011±71 *c*	187±31 *a*	45±9 *cb*	0.01±0.00 *a*
Pinto	532±52 *b*	1774±41 *d*	171±15 *ab*	38±1 *c*	0.01±0.00 *a*
Mean	662±93	2218±258	168±28	52±13	0.01±0.00
Chickpea					
Desi	340±51 *b*	1437±58 *b*	113±24 *a*	55±10 *a*	2±2 *a*
Kabuli	543±48 *a*	1629±6 *a*	127±39 *a*	25±6 *a*	9±6 *a*
Mean	441±120	1533±112	120±30	40±18	5±6

Data represent mean value ± standard deviation. Values are presented on a wet weight basis (10% moisture). Values within each market class followed by a different letter are significantly different at $P < 0.05$ (n=42).

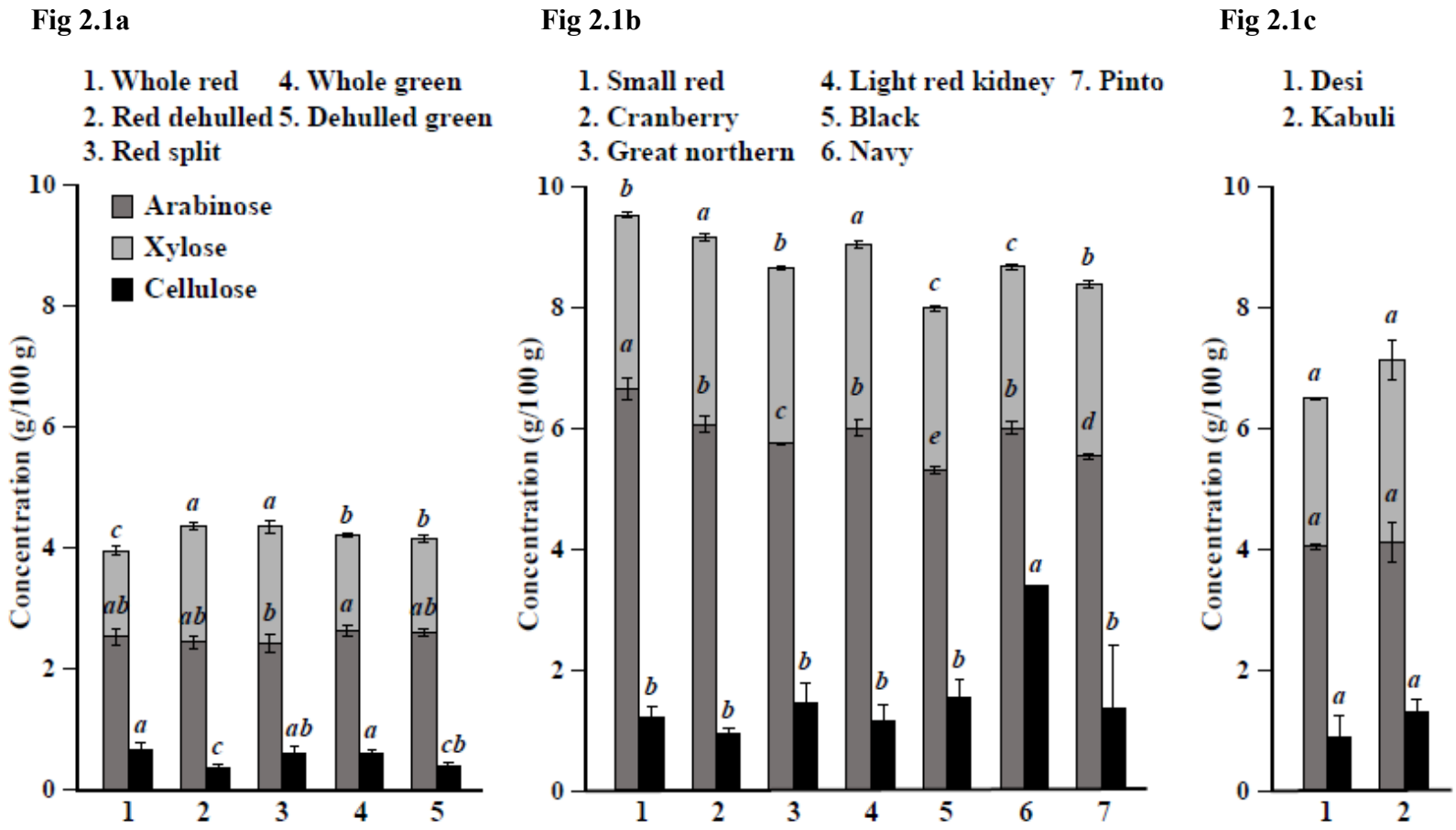

Figure 2. 1. Hemicellulose (arabinose+xylose) and cellulose concentrations in different a) lentil, b) common bean, and c) chickpea market classes. Values are presented on a wet weight basis (10% moisture). Values within each market class followed by a different letter are significantly different at $P < 0.05$ (n=42).

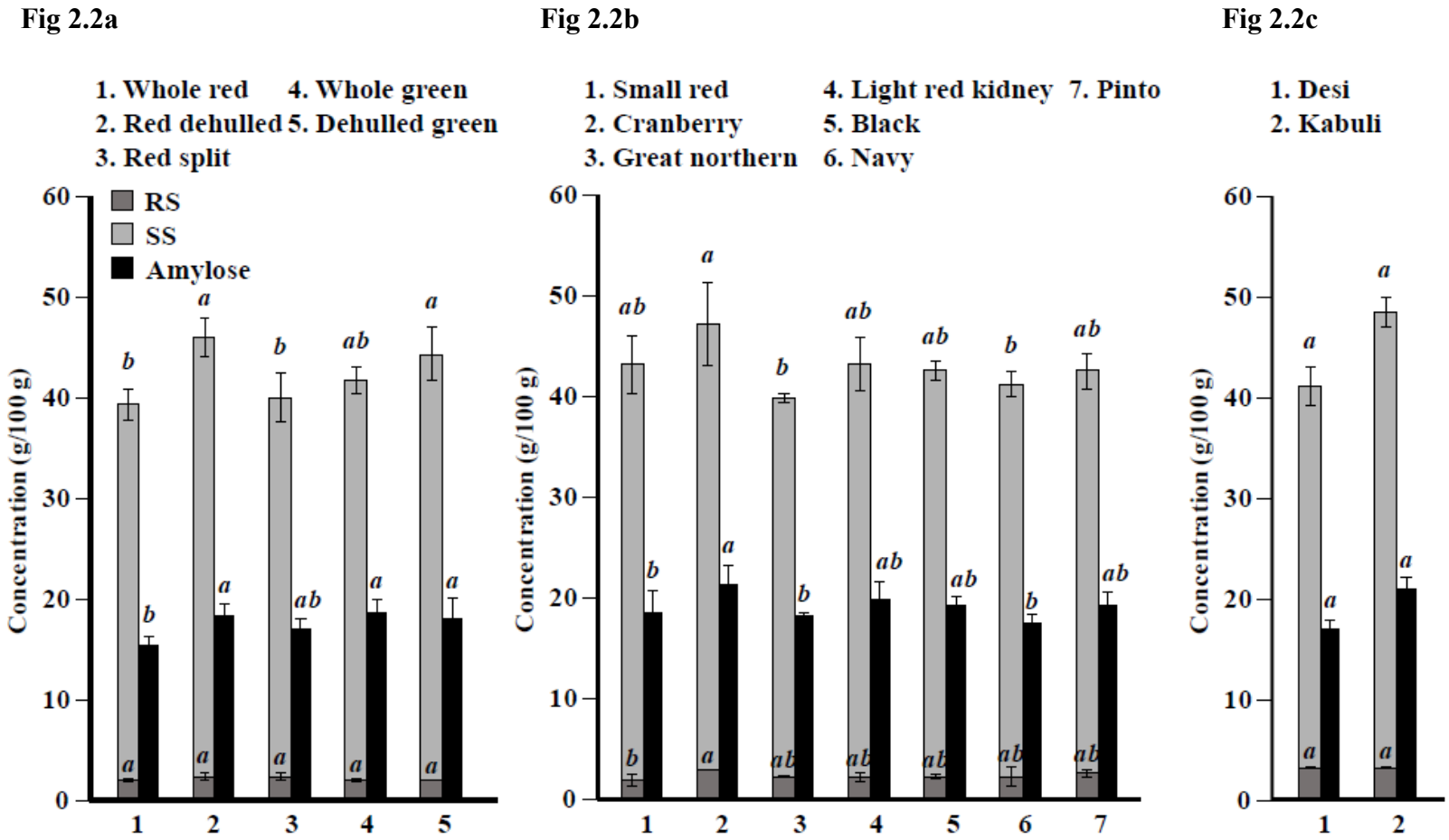

Figure 2. 2. Soluble starch (SS), resistant starch (RS), and total amylose concentration in different a) lentil, b) common bean, and c) chickpea market classes. Values are presented on wet weight basis (10% moisture). Values within each market class followed by a different letter are significantly different at $P < 0.05$ (n=42).

sorbitol and xylitol concentrations. Considering common bean RFO, stachyose concentrations were higher (1.8-2.5 g/100 g) than those for raffinose (532-754 mg/100 g) and verbascose (128-187 mg/100 g) **(Table 2.5)**. For FOS, kestose concentrations (38-69 mg/100 g) were higher than nystose concentrations (0.01-0.01 mg/100 g) **(Table 2.5)**. Common bean arabinose and xylose concentrations ranged from 5.3-6.6 g/100 g and 2.7-3.1 g/100 g, respectively **(Figure 2.1b)**. Among common bean market classes, small red had significantly more ($P < 0.05$) arabinose and cranberry bean and light red kidney bean had significantly more ($P < 0.05$) xylose. Cellulose concentrations ranged from 0.9 to 3.4 g/100 g, with navy bean having the highest concentration **(Figure 2.1b)**. Soluble starch, RS, and amylose concentrations ranged from 38-44, 2-3, and 18-21 g/100, respectively. Overall, cranberry bean had higher SS, RS, and amylose concentrations **(Figure 2.2b)**.

2.6.3. Chickpea

Sucrose was the most abundant simple sugar (1.8-2.5 g/100 g) in chickpea, followed by glucose (30-32 mg/100 g), fructose (2.2-2.5 mg/100 g), mannose (0.5-0.8 mg/100 g), and rhamnose (0.1-0.1 mg/100 g) **(Table 2.3)**. Among chickpea SAs, sorbitol concentrations (473-557 mg/100 g) were higher than mannitol (15-19 mg/100 g) and xylitol (14-18 mg/100 g) concentrations **(Table 2.4)**. Overall, desi had higher sorbitol, mannitol, and xylitol concentrations than kabuli; however, differences were only significant for sorbitol and xylitol ($P < 0.05$). Among RFO in chickpea, stachyose concentrations (1.4-1.6 g/100 g) were higher than raffinose (340-543 mg/100 g) and verbascose (113-127 mg/100 g) concentrations **(Table 2.5)**. Kabuli had significantly more ($P < 0.05$) raffinose and stachyose than desi. Considering FOS in chickpea, kestose

concentration (25-55 mg/100 g) was higher than nystose concentration (2-9 mg/100 g) **(Table 2.5)**. Arabinose, xylose, cellulose, SS, RS, and amylose concentrations ranged from 4.0-4.1, 2.5-3.0, 0.9-1.3, 38-45, 3.1-3.1, and 17-21 g/100 g, respectively, but none of these were significantly different between desi and kabuli **(Figures 2.1c and 2.2c)**.

2.7. Discussion

Pulses, including lentil, common bean, and chickpea, are traditional staple foods that have been consumed for several centuries because of their superior nutritional profile (Johnson et al., 2013; Sen Gupta et al., 2013; Thavarajah et al., 2011; Wang et al., 2009). However, increasing global demand for highly processed sugar and fat-rich foods has led to severe non-communicable disease epidemics, including obesity, overweight, and cancer (Mitchell et al., 2009). A diet rich in prebiotic carbohydrates, low in energy and glycemic response, moderate in protein, low in fat, and rich in micronutrients is now recommended for weight management (WHO, 2014). Cereal-based diets can satisfy daily caloric requirements, but do not provide daily requirements of prebiotic carbohydrates in a single serving (Williams, 1995). The present study indicates that pulses (lentil, common bean, and chickpea) provide 60 to 75% of the daily safe requirement of prebiotic carbohydrates (20 g/day) in a single serving **(Table 2.2;** Douglas and Sanders, 2008**)**. The official recommendations have not been made yet for prebiotic carbohydrate consumption, however several researches have offered suggestions for safe intake (Douglas and Sanders, 2008). Additionally, this current work provides information on the types and quantities of prebiotic carbohydrates in - different pulse market classes,

which is valuable for further enhancement of nutritional quality via plant breeding and genetic selection.

Simple sugar concentrations in lentil, common bean, and chickpea are comparable to previous studies (Sánchez-Mata et al., 1998). Simple sugar concentrations in common bean were higher than in lentil and chickpea. In contrast, SA concentrations were higher in lentil and chickpea than in common bean. Simple sugars are precursors of SA formation in plants; however, this negative correlation between simple sugars and SA is largely dependent on plant type and weather conditions (Krasensky and Jonak, 2012). Simply, from 5.1 to 6.7, 1.7 to 2.6, and 2.1 to 2.8 g/100 g for RFO (Gangola et al., 2016; Johnson et al., 2015b; Reddy et al., 1984) and 0.0 to 0.7, 0.0 to 0.5, and 0.0 to 0.07 g/100 g for FOS (Biesiekierski et al., 2011; Johnson et al., 2015a) in lentil, common bean, and chickpea, respectively. These values are comparable to those from the current study. Further, the present study found total polysaccharides are higher in common bean and chickpea than in lentil, similar to previous reports (Dodevska et al., 2013; Singh, 1984). The composition of carbohydrates depends on their localization in the seed coat or cotyledon (Guillon and Champ, 2002). Cell walls of the cotyledon contain a range of polysaccharides including cellulose, starch, and non-starchy non-cellulosic glucans, while the seed coat contains large quantities of low molecular weight carbohydrates and cellulose but is low in hemicellulose (Guillon and Champ, 2002). Lentil seeds are generally smaller than common bean and chickpea **(Table 2.1)**; this might explain why increased levels of low molecular weight carbohydrates (SA, RFO, and FOS) are found

in lentil while common bean and chickpea contain higher levels of cellulose and hemicellulose **(Table 2.2)**.

Sucrose is the most abundant simple sugar found in pulses. During the development of the endosperm in the seed, the concentration of hexose declines while sucrose increases (Hill et al., 2003). Among lentil market classes, red lentil has higher levels of simple sugars than green lentil. Also, whole green lentil (lentil with seed coat) contains more sucrose, glucose, and fructose than dehulled green lentil, in accordance with earlier studies (Wang, 2008; Wang et al., 2009) the opposite is true with respect to mannose **(Table 2.3)**. In common bean, cranberry, small red, and great northern bean had higher total simple sugars while black and navy bean had the least **(Table 2.3)**, showing significant variation among market classes due to structural (i.e., seed size), genetic, and environmental variations (Reddy et al., 1984). Among chickpea market classes, kabuli had significantly more sucrose than desi due to its larger cotyledon size (Wang and Daun, 2004).

With respect to SAs, whole red lentil had higher sorbitol than dehulled lentil and dehulled red lentil had higher mannitol and xylitol; however, the opposite is true for green lentil, showing that SA distribution in lentil seed is influenced by both market class (red vs. green) and processing method (whole vs. dehulled), as noted previously (Siva et al., 2018). Common bean market classes also varied with respect to SA levels and had more mannitol and xylitol than sorbitol. Light red kidney bean, which has the largest seed size among studied market classes, had 50% more SA than all other market classes. In chickpea, desi (smaller seed size, and hence more seed coat area) had more SA than

kabuli, which is attributed to the more SA being present in seed coat than the cotyledon. Across all three pulse crop types, SA varied with seed size, market class, and processing method.

Lentil RFO concentration varies with genotype and growing environment (Johnson et al., 2013, 2015a). Moreover, dehulling generally reduces raffinose concentrations but increases stachyose and verbascose concentrations (Johnson et al., 2015b; Siva et al., 2018; Wang et al., 2009). In the current study, dehulling only increased verbascose concentration in red lentil. The greater variation in stachyose vs. raffinose and verbascose levels among common bean market classes might be due to genetic differences. Along with variations in seed size, seed coat thickness, and surface area, genetic makeup might affect the RFO concentration in common bean. In chickpea, more RFOs were found in kabuli (Wang and Daun, 2004), which has a large seed size and hence a larger seed cotyledon **(Tables 2.1 and 2.5)**. With respect to FOS, present data show higher levels of kestose present in the seed cotyledon than the seed coat in red lentil, with the reverse observed in green lentil **(Table 2.5)**. Kestose levels varied significantly among common bean and chickpea market classes, indicating that kestose synthesis might be influenced by market class (Patrick et al., 2013).

The seed coat contains most of the cellulose found in the seed (Bhattacharya et al., 2005). Present data confirm that whole lentil generally had higher cellulose levels than dehulled lentil. Similarly, arabinose and xylose were slightly higher in whole lentil and dehulled lentil, respectively, reflecting differences in the distribution of hemicellulose compounds in the seed. Cellulose levels are higher in common bean

market classes when the seed size decreases, suggesting cellulose compounds are abundant in the seed coat. In contrast, arabinose and xylose levels are positively correlated with seed size. In chickpea, significant differences between desi and kabuli were not observed, which contrasts with previously reported results (Singh, 1984).

The RS levels of raw pulses were ranged from 3 to 21 g/100 g in previous studies (García-Alonso et al., 1998; de Almeida Costa et al., 2006; Johnson et al., 2013; Johnson et al., 2015b). High variation in the RS yield may due to differences in the RS analysis methods, particle size of the pulse flour, and the genetic and environmental variation of the pulses. In present study, lentil dehulling slightly increases RS and SS as dehulling removes the starch-free seed coat, therefore concentrating starch fractions in the seed cotyledon (Johnson et al., 2015b; Siva et al., 2018). In common bean and chickpea market classes, RS and SS are positively correlated with seed size **(Figure 2.2b, Table 2.1)**, which relates to where starch compounds are stored in the cotyledon. Further, data from the current study confirm the positive correlation of amylose concentrations with RS, SS, and total starch (sum of RS and SS), similar to previous reports (Yadav et al., 2009). Johnson et al. (2015b) indicated that significant changes in lentil RS concentration due to processing, cooking, and cooling. Cooling of cooked lentil increased RS concentration approximately two-fold from 3.0 % (w/w) in cooked lentil to 5.5 % (w/w) after cooling. Further, RS concentrations ranged from 3 - 5% (w/w) in raw lentil and the concentrations of RS in raw and cooked lentils were not significantly different (Johnson et al., 2015). This current study reports only dry pulse seed RS concentrations for future breeding and selection purposes.

Overall, prebiotic carbohydrates represented 24, 28, and 22% of the total carbohydrate compounds in lentil, common bean, and chickpea, respectively. Prebiotic carbohydrate concentrations differ among pulses due to seed size, type of pulse, and processing method, and therefore incorporation of several pulses in the diet provides a range of different prebiotic carbohydrates needed for gut health. However, this present study did not report several prebiotic carbohydrates including pectin, and types of hemicellulose which does occur in most legume seeds. Further, complete profiling of carbohydrates in pulses provides useful information for future plant breeding and genetic studies to understand the prebiotic carbohydrate control mechanism in plants (Vinocur and Altman, 2005).

2.8. Conclusion

This study shows the type and quantity of prebiotic carbohydrates varies with pulse crop, market class, seed size, and processing method. Lentil, common bean, and chickpea provide 60-75% of the suggested daily intake of prebiotic carbohydrates in a 100 g serving. Lentil is rich in low molecular weight carbohydrates including SA, RFO, and FOS, while common bean and chickpea are rich in polysaccharides such as cellulose, hemicellulose, and amylose. Overall, these pulses are rich in prebiotic carbohydrates, and further nutritional breeding is possible with identifying suitable growing locations, and genotypes producing higher levels of prebiotic carbohydrates in different pulse crop market classes.

2.9. Acknowledgements

Funding support for this project was provided by the Plant Health and Production and Plant Products: Plant Breeding for Agricultural Production program area [grant no. 2018-67014-27621/project accession no. 1015284] of the USDA National Institute of Food and Agriculture, the International Center for Dry Land Agriculture (ICARDA, Morocco), and the American Pulse Association.

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This chapter was published in Frontiers in Nutrition; Siva, N., Thavarajah, P., Kumar, S., and Thavarajah, D. (2019). Variability in Prebiotic Carbohydrates in Different Market Classes of Chickpea, Common Bean, and Lentil Collected From the American Local Market. Front. Nutr. 6, 38. doi:10.3389/fnut.2019.00038.

3. CHAPTER TWO

PREBIOTIC CARBOHYDRATE CONCENTRATIONS OF COMMON BEAN AND CHICKPEA CHANGE DURING COOKING, COOLING, AND REHEATING

3.1. Hypotheses

H_0: Cooking, cooling, and reheating do not change the concentrations of SA, RFO, FOS, RS, and amylose concentration in common bean and chickpea market classes.

H_1: Cooking, cooling, and reheating change the concentrations of SA, RFO, FOS, RS, and amylose in common bean and chickpea market classes.

3.2. Objective

Determine changes of SA, RFO, FOS, RS, and amylose concentration in seven common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto) and two chickpea market classes (desi and kabuli) in response to cooking, cooling, and reheating.

3.3. Abstract

Thermal processing of pulse crops influences the type and levels of prebiotic carbohydrates present. Pulses such as common bean and chickpea are rich sources of prebiotic carbohydrates including sugar alcohols (SAs), raffinose family oligosaccharides (RFOs), fructooligosaccharides (FOSs), resistant starch (RS), and amylose. This study determined changes in prebiotic carbohydrate concentrations of seven common bean and two chickpea market classes after thermal processing (cooking, cooling, reheating). A

100-g serving of common bean provides 0.7–10.6 mg of SAs, 3.9–5.2 g of RFOs, 57–143 mg of FOSs, 2.6–3.9 g of RS, and 25–33 g of amylose; cooling and reheating reduced RFOs but increased SAs, FOSs, and RS in many cases. A 100-g serving of chickpea provides 1.2–1.7 g of SAs, 2.5–3.2 g of RFOs, 26–43 mg of FOSs, 3.6–5.3 g of RS, and 24–30 g of amylose; cooling and reheating reduced SAs and RFOs but increased FOSs, RS, and amylose concentrations. Processing methods change the nutritional quality of pulse crops by changing the type and quantity of prebiotic carbohydrates.

Keywords: Pulse crops, food processing, sugar alcohols, raffinose family oligosaccharides, fructooligosaccharides, resistant starch, amylose

3.4. Introduction

Pulses are becoming popular in Western diets due to increased awareness of their nutritional benefits (Hall et al., 2017). Prebiotic carbohydrates benefit human health as they are fermented by favorable gut microorganisms (Gibson et al., 2017); reduce cholesterol formation, body cell inflammation, and blood pressure; and increase satiety hormones and nutrient absorption by changing the gut pH (Joshi et al., 2018). Two types of prebiotic carbohydrates are generally present in plant-based foods: low molecular weight carbohydrates include sugar alcohols (SAs: sorbitol and mannitol), raffinose family oligosaccharides (RFOs: raffinose, stachyose, and verbascose), and fructooligosaccharides (FOSs: kestose and nystose); and high molecular weight carbohydrates include cellulose, hemicellulose, inulin, and resistant starch (RS) (Roberfroid et al., 2010). Common bean (Phaseolus vulgaris L.) and chickpea (Cicer arietinum L.) respectively contain 0.4–5.6 and 1.2–2.9/100 g of RFOs (Gangola et al.,

2016; Reddy et al., 1984). Further, these pulse crops contain 0–1 and 0–0.07 g/100 g of FOSs (Biesiekierski et al., 2011) and 2.9–4.5 and 2.4–4.4 g/100 g of RS, respectively (de Almeida Costa et al., 2006).

Prebiotic carbohydrate concentrations and nutritional benefits change during processing. Cooking and microwave reheating of lentil can reduce RFOs (from 5.5–6.1% to 4.3–4.9%) and cooling (at 4 °C for 24 h) can cause a twofold increase in RS (Johnson et al., 2015; Siva et al., 2018). Processing leads to structural changes of starch molecules—e.g., amylose:amylopectin ratio, amylose retrogradation, amylose chain length, linearization of amylopectin—in response to temperature, water content, and pressure (Sajilata et al., 2006). These structural changes vary with the physical properties (size and seed coat thickness) and chemical composition (protein, fat content, amylose:amylopectin ratio, minerals, and endogenous enzymes) of the seed (Varatharajan et al., 2011; Wang et al., 2003).

Seed physical properties influence water absorption and heat diffusion during processing (Kumar et al., 2018; Shafaei et al., 2016; Turhan et al., 2002). The seed size of common bean varies from 20–52 g/100 seeds (White and González, 1990). Common bean market classes also demonstrate different seed volume, seed density, hydration, and swelling capacity (Shimelis and Rakshit, 2005). As a result, the cooking time of common bean ranges from 19–34 min (Shimelis and Rakshit, 2005). The seed size of chickpea market classes varies from 14–32 g/100 seeds (Hossain et al.; Kaur et al., 2005). Kabuli chickpea has a higher seed volume, seed density, and hydration capacity than desi chickpea. Therefore, kabuli requires a longer cooking time (93–97 min) than the desi type

(62–78 min) (Kaur et al., 2005). Overall, the physical differences of pulse crops influence the thermal processing time, which may lead to thermal degradation of inherent nutrients including prebiotic carbohydrates.

Cooked pulse seeds have different prebiotic carbohydrate types and concentrations than raw seeds (Chung et al., 2008; Du et al., 2014). In a pair of studies, black, pinto, and navy bean had higher concentrations of RS than red kidney bean before cooking, but red kidney bean had significantly higher levels of RS than all other market classes after cooking (Du et al., 2014a; Du et al., 2014b). The authors speculated the differences in RS concentration in red kidney bean were related to starch granule size, amylose concentration, and water absorption. Similar results have been reported for raw desi chickpea, which had higher RS than kabuli due to its smaller seed size and higher amylose concentration (Chung et al., 2008; Miao et al., 2009; Wang et al., 2010). Further, cooking can reduce the RFO levels in both common bean and chickpea (Wang et al., 2010).

Limited data are available on corresponding changes of SAs, FOSs, and amylose in common bean and chickpea market classes and their impact on human nutrition. Yet, comparison of prebiotic carbohydrate types and concentrations in thermally processed pulse seeds is vital information for making nutritional composition recommendations towards new food product development. This study was designed to determine changes of SAs, RFOs, FOSs, RS, and amylose concentration in seven common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto) and

two chickpea market classes (desi and kabuli) in response to cooking, cooling, and reheating.

3.5. Materials and Methods

3.5.1. Materials

Chemicals and enzymes were purchased from Fisher Scientific (Asheville, NC, USA), Sigma-Aldrich (St. Louis, MO, USA), and VWR International (Suwanee, GA, USA). Water, distilled and deionized (ddH_2O) to a resistance of ≥ 18.2 MΩ (NANO-pure Diamond, Barnstead, IA, USA), was used to make solvents and dilutions.

3.5.2. Seed samples

Approximately 1-kg samples of seven commercially available common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto) and two chickpea market classes (desi and kabuli) were obtained from a commercial distributor (AGT Foods, Bismarck, ND, USA) **(Table 3.1)**. Samples were homogenized by mixing, subsampled, and then stored at ambient temperature (20–25 °C). The treatment design was a completely randomized design with seven common bean types and two chickpea types (n=9), three food processing methods (cooking, cooling, and reheating) (n=3), and three replicates (n=3). The experiment was conducted two times for a total of 162 samples.

3.5.3. Seed size

One hundred (100) seeds were counted and then weighed using an electronic balance (model B1240, American Scientific Products, Japan), with data expressed as the weight (g) of 100 seeds.

Table 3. 1. Physical characteristics of pulse market classes used in this experiment.

Type	Market class	Seed size (g/100 seeds)	Water absorption[a] (g/100 g seeds)	Seed coat thickness (mg/cm^2)
Common bean	Small red	31-33	20-24	11-12
	Cranberry	56-57	10-12	21-24
	Great northern	30-37	42-44	16-19
	Light red kidney	59-63	28-41	20-21
	Black	18-20	69-88	19-22
	Navy	19-21	74-80	12-14
	Pinto	34-36	27-31	19-20
Chickpea	Desi	23-25	61-73	22-23
	Kabuli	47-49	57-64	7-8

[a] over 4 h.

3.5.4. Water absorption

Five grams of seeds were soaked in 15 g of water at room temperature for 4 h. Seed samples were then drained, blotted with tissue paper, and weighed, with data expressed as the amount of water (g) absorbed per 100 g of seeds (AACC International, 2010).

3.5.5. Seed coat thickness

Seed coats were removed using a mortar and pestle, with data expressed as coat weight (mg) per unit area (1 cm^2) (Gil et al., 1996).

3.5.6. Cooking, cooling, and reheating

Three replicates of samples (6 g) were placed in 50-mL Pyrex® beakers with ddH_2O at a weight ratio of 1:3 (seeds:water). The samples were placed in a slow cooker (Model 33156SZ, Hamilton Beach Brands, Inc., Glen Allen, VA) and cooked for 4 h. Similar set of samples were cooked and stored at room temperature for 1 h and then refrigerated (ROPER, Whirlpool Corporation, MI, USA) at 4 °C for 24 h. Third set of

samples were cooked, cooled, and reheated in a microwave oven (General Electronic Co., Louisville, KY, USA) at high power (950 W) for 1 min. The entire experiment was duplicated. Cooked, cooled, and reheated samples were homogenized, and their moisture content measured by a gravimetric method (AACC International, 2010). Data are reported on a wet weight basis (normalized to 15% moisture).

3.5.7. Low molecular weight carbohydrates (LMWCs)

LMWCs were extracted from 500-mg samples as per Muir et al., 2009. SA, RFO, and FOS concentrations were measured using high-performance anion exchange chromatography (HPAE) (Dionex, ICS-5000, Sunnyvale, CA, USA) (Feinberg et al., 2009) connected to a CarboPac PA1 column (250 × 4 mm; Dionex, CA, USA) and a CarboPac PA1 guard column (50 × 4 mm; Dionex, CA, USA). The mobile phases used were 100 mM sodium hydroxide/600 mM sodium acetate (A), 200 mM sodium hydroxide (B), and ddH_2O (C) with a flow rate of 1 mL/min as described by Johnson et al., 2013. Detection was carried out using a pulsed amperometric detector (PAD; ICS-5000, Thermo Scientific, USA) with a gold electrode and a silver-silver chloride reference electrode at 2.0 μA. LMWC concentrations were detected within a linear range of 3 to 1000 μg/g, with a minimum detection limit of 0.2 μg/g. An external reference (CDC Redberry lentil) was used to ensure the accuracy and reproducibility of the detection. Peak areas of the external reference, glucose (100 ppm), SAs (3–1000 ppm), RFOs (3–1000 ppm), and FOSs (3–1000 ppm) were routinely analyzed for method consistency and detector sensitivity with an error of less than 5% (Johnson et al., 2013). The concentration of LMWCs in the samples (C_s) was calculated according to $C_s = (C_f \times$

V) / m, where C_f is the filtrate concentration obtained from HPAE, V is the final diluted volume, and m is the mass of the sample (moisture corrected).

3.5.8. Resistant Starch (RS)

RS concentration was measured using α-amylase and amyloglucosidase enzymes as previously described (McCleary and Monaghan, 2002). The glucose concentration (C_G) resulting from enzymatic hydrolysis of resistant starch was measured using HPAE-PAD. RS concentration (C_{RS}) was calculated according to $C_{RS} = (C_G \times 0.9 \times V) / m$, where 0.9 is a factor to convert free glucose to anhydrous glucose as occurs in starch (McCleary and Monaghan, 2002), V is the final diluted volume, and m is the mass of the sample (moisture corrected). Regular corn starch (RS concentration 1.0±0.1% (w/w)) was used to validate the data. Batches were checked regularly to ensure an analytical error of less than 10%.

3.5.9. Amylose concentration

Seed amylose concentration was measured as previously described (Gibson et al., 1997) using an α-amylase enzyme (67 U/mL) and amyloglucosidase (333 U/mL). The glucose concentration resulting from enzymatic hydrolysis of amylose and total starch fractions was measured using a glucose oxidase and peroxidase (GOPOD) method. Absorbance was measured at 510 nm in a spectrophotometer and amylose concentration was calculated as follows:

$$\text{Amylose (\%)} = \frac{\text{Abs}_{\text{(Con A supernatant)}}}{\text{Abs}_{\text{(Total starch aliquot)}}} \times \frac{6.15}{9.2} \times 100,$$

$$\text{Amylose (g/100 g)} = \text{Total starch concentration} - \frac{\text{Amylose (\%)}}{100},$$

where 6.15 and 9.2 are dilution factors.

3.5.10. Scanning electron microscopy (SEM)

Freshly cooked, cooled, and reheated pinto bean and kabuli chickpea samples were prepared for SEM imaging. Seeds and razor blades were submerged in liquid nitrogen until completely frozen. A fraction of the seed cotyledon was then cut with the frozen razor blade and attached to a multi-brace holder with carbon tape. The brass holder was inserted into a variable pressure scanning electron microscope (S-3400N, Hitachi High-Technologies, Tokyo, Japan) under low vacuum. The samples were allowed to stand for 10-15 min until the surface moisture sublimated. Backscattered electron images of each sample were taken within 15 min using an accelerating voltage of 8 kV and a chamber pressure of 30 Pa.

3.5.11. Statistical analysis

Common bean and chickpea market classes, processing methods, runs, and replicates were considered as random factors and class variables. Analysis of variance (ANOVA) was performed using the General Linear Model procedure (PROC GLM) of SAS 9.4 (SAS Institute Inc., 2017). Fisher's protected least significant difference (LSD) at $P < 0.05$ was used to separate means.

3.6. Results

Seed size, water absorption, and seed coat thickness varied among common bean and chickpea market classes **(Table 3.1)**. For common bean, black had the smallest seed size (18–20 g/100 seeds) and highest water absorption (69–88 g/100 g seeds). Cranberry had a slightly smaller seed size (56–57 g/100 seeds) compared to light red kidney (59–63 g/100 seeds) but a greater seed coat thickness (21–24 mg/cm^2) and the lowest water absorption

(10–12 g/100 g seeds). For chickpea, kabuli had a larger seed size (47–49 g/100 seeds) with lower water absorption and seed coat thickness than desi **(Table 3.1)**.

Total prebiotic carbohydrates (sum of SAs, RFOs, FOSs, and RS) in cooked common bean ranged from 6.8 to 8.3 g/100 g **(Table 3.2)**. The concentration of total prebiotic carbohydrates decreased significantly ($P < 0.05$) after cooling and reheating in small red and pinto bean. For chickpea, total prebiotic carbohydrates ranged from 8.0 to 9.7 g/100 g after cooking but then after reheating significantly declined in desi but increased in kabuli ($P < 0.05$) **(Table 3.2)**. Overall, common bean and chickpea respectively provide 36–39% and 45–49% of the daily recommended safe intake of prebiotic carbohydrates.

Total SA concentrations ranged from 0.7–10.6 mg/100 g for common bean and 1197–1709 mg/100 g for chickpea after cooking **(Table 3.3)**. Sorbitol concentration varied from 0.5–2.2 mg/100 g after cooking for common bean but significantly ($P < 0.05$) increased after cooling and reheating in cranberry, great northern, and pinto bean. Mannitol concentration ranged from 0.10–0.42 mg/100 g for cooked common bean but significantly ($P < 0.05$) decreased after cooling and reheating in all market classes except black bean **(Table 3.3)**. For chickpea, sorbitol concentration after cooking ranged from 984–1358 mg/100 g and mannitol concentration ranged from 213–351 mg/100 g. Cooling did not change sorbitol or mannitol levels in either chickpea type but reheating significantly decreased ($P < 0.05$) mannitol levels **(Table 3.3)**.

Total RFOs ranged from 3.9–5.2 g/100 g for common bean and 2.5–3.2 g/100 g for chickpea after cooking **(Table 3.4)**. Common bean had a higher concentration of

Table 3. 2. Concentrations of total prebiotic carbohydrates (g) in a 100 g serving of cooked, cooled, and reheated common bean and chickpea market classes with percent recommended dietary allowance.

Market classes	Total prebiotic carbohydrates* (g/100 g)			Recommended dietary allowance (%)		
	Cooked	Cooled	Reheated	Cooked	Cooled	Reheated
Common beans						
Small red bean	8.2±0.3a	7.2±0.3b	5.5±0.3c	41±1a	36±1b	28±2c
Cranberry bean	7.6±0.9a	7.3±0.5a	6.2±0.9b	38±4a	37±2a	31±4b
Great northern bean	6.8±1.1a	7.2±0.8a	7.0±1.0a	34±5a	36±4a	35±5a
Light red kidney bean	8.0±0.7a	7.4±1.0a	7.4±2.5a	40±3a	37±5a	37±12a
Black bean	8.3±0.7a	8.1±0.5ab	7.6±0.9b	42±3a	41±2ab	38±4b
Navy bean	8.0±0.3a	7.9±0.4a	7.7±1.5a	40±1a	40±2a	39±8a
Pinto bean	6.9±0.7a	7.4±1.4b	8.3±2.1a	35±3b	37±7b	42±11a
Mean	7.7±0.9a	7.5±0.8a	7.1±1.7b	39±4a	38±4a	36±8b
Chickpea						
Desi	8.0±0.8a	7.5±0.6ab	7.4±1.6b	40±4a	38±3ab	37±8b
Kabuli	9.7±0.7b	11.8±1.8a	11.1±1.1a	49±3a	59±9a	56±6a
Mean	8.9±1.2b	9.7±2.5a	9.3±2.4ab	45±6b	49±13a	47±12ab

*Total prebiotic carbohydrates were calculated by adding concentrations of sugar alcohols, raffinose family oligosaccharides, fructooligosaccharides, and resistant starch. Recommended dietary allowance of prebiotic carbohydrates is 20 g/d (Douglas & Sanders, 2008).

Table 3. 3. Sugar alcohol (SA) concentrations (mg/100 g) in common bean and chickpea market classes after cooking, cooling, and reheating.

SAs	Common bean							Chickpea	
	srb[a]	cb	gnb	lrkb	bb	nb	pb	desi	kabuli
Sorbitol									
Cooked	1.5±0.8a	1.2±0.5c	0.5±0.4c	10.2±6.9a	2.2±1.0a	0.9±0.6a	1.8±0.4b	1358±74a	984±42a
Cooled	1.8±1.5a	4.2±4.1b	1.0±1.0b	9.2±4.0a	1.5±0.8b	1.5±1.6a	2.4±1.5b	1270±51a	946±37a
Reheated	1.9±1.6a	5.7±5.6a	3.7±0.4a	10.1±3.6a	2.0±1.0ab	1.4±1.2a	4.3±1.4a	1319±159a	942±169a
Mannitol									
Cooked	0.3±0.1a	0.18±0.04a	0.16±0.02a	0.42±0.04a	0.21±0.20ab	0.17±0.10a	0.10±0.04a	351±71a	213±21a
Cooled	0.2±0.2b	0.06±0.04c	0.08±0.07c	0.33±0.11b	0.14±0.18b	0.14±0.08b	0.08±0.04b	324±73a	231±48a
Reheated	0.2±0.1b	0.12±0.02b	0.12±0.09b	0.26±0.06b	0.28±0.22a	0.08±0.06c	0.06±0.04b	226±246b	135±118b
Total SAs									
Cooked	1.8±0.7a	1.5±0.5c	0.7±0.4c	10.6±6.9a	2.4±1.2a	1.1±0.7a	1.9±0.4b	1709±40a	1197±51a
Cooled	1.9±1.4a	4.3±4.1b	1.1±0.9b	9.6±3.9a	1.6±1.0b	1.6±1.7a	2.4±1.5b	1594±108ab	1177±43a
Reheated	2.0±1.4a	5.9±5.6a	3.8±0.4a	10.4±3.7a	2.2±1.2ab	1.5±1.2a	4.4±1.4a	1545±379b	1076±105b

[a]Mean (±standard deviation) values within a column followed by a different letter are significantly different at $P < 0.05$ ($n = 162$). Values were calculated on a wet weight basis (15% moisture). Small red bean (srb), cranberry bean (cb), great northern bean (gnb), light red kidney bean (lrkb), black bean (bb), navy bean (nb), and pinto bean (pb).

Table 3. 4. Raffinose family oligosaccharide (RFO) concentrations in common bean and chickpea market classes after cooking, cooling, and reheating.

RFOs	Common bean							Chickpea	
	srb[a]	cb	gnb	lrkb	bb	nb	pb	desi	kabuli
Raffinose (g/100 g)									
Cooked	1.0±0.2a	1.1±0.2a	1.0±0.2a	1.3±0.2a	1.3±0.1a	1.2±0.1a	1.1±0.1b	0.6±0.1a	0.8±0.2a
Cooled	1.0±0.1ab	1.0±0.1a	0.9±0.1a	1.2±0.0b	1.3±0.1a	1.0±0.1b	1.0±0.0c	0.6±0.1a	0.7±0.2a
Reheated	0.8±0.1b	1.1±0.2a	1.1±0.1a	1.0±0.1c	1.2±0.1a	1.0±0.1b	1.2±0.2a	0.6±0.1a	0.7±0.2a
Stachyose (g/100 g)									
Cooked	3.2±0.4a	3.0±0.5a	2.8±0.6a	3.6±0.5a	3.8±0.4a	3.4±0.4a	3.0±0.2a	1.8±0.1a	2.3±0.2a
Cooled	2.8±0.3b	2.6±0.7b	2.9±0.8a	3.4±1.0b	3.6±0.6a	3.0±0.6b	2.7±0.7b	1.6±0.2b	2.2±0.3a
Reheated	2.4±0.6c	2.6±0.7b	2.9±0.6a	2.3±0.6c	2.8±0.5b	2.3±0.3c	2.8±0.8b	1.6±0.5b	1.9±0.2b
Verbascose (mg/100 g)									
Cooked	69±27a	128±20a	47±4b	118±11a	78±7b	72±9a	62±12b	31±20b	49±16b
Cooled	70±19a	103±18a	66±14b	96±28b	102±14a	87±23a	89±16a	42±24b	50±14b
Reheated	70±19a	132±15a	101±23a	104±33ab	113±5a	83±18a	101±16a	63±22a	63±25a
Total RFOs (g/100 g)									
Cooked	4.3±0.3a	4.2±0.5a	3.9±0.8a	4.9±0.4a	5.2±0.5a	4.8±0.4a	4.2±0.2a	2.5±0.1a	3.2±0.2a
Cooled	3.9±0.2b	3.7±0.7b	3.9±0.9a	4.6±0.9b	5.0±0.6a	4.1±0.5b	3.8±0.7b	2.2±0.2b	3.0±0.1a
Reheated	3.3±0.8c	3.8±0.9ab	4.1±0.7a	3.4±0.7c	4.1±0.6b	3.4±0.3c	4.1±1.0a	2.2±0.6b	2.7±0.2b

[a]Mean (±standard deviation) values within a column followed by a different letter are significantly different at $P < 0.05$ ($n = 162$). Values were calculated on a wet weight basis (15% moisture). Small red bean (srb), cranberry bean (cb), great northern bean (gnb), light red kidney bean (lrkb), black bean (bb), navy bean (nb), and pinto bean (pb).

stachyose (2.8–3.8 g/100 g) followed by raffinose (1.0–1.3 g/100 g) and verbascose (47–128 mg/100 g) **(Table 3.4)**. Raffinose and stachyose concentrations decreased significantly ($P < 0.05$) after cooling and reheating in four of the six common bean market classes. Cooked chickpea had higher levels of stachyose (1.8–2.3 g/100 g) than raffinose (0.6–0.8 g/100 g) and verbascose (31–49 mg/100 g). A slight reduction was observed in raffinose concentration after processing in kabuli, but the differences were not significant. Stachyose concentration decreased and verbascose levels increased significantly ($P < 0.05$) after cooling and reheating in both chickpea types **(Table 3.4)**.

Total FOSs ranged from 57–143 mg/100 g for common bean and 26–43 mg/100 g for chickpea market classes after cooking **(Table 3.5)**. For common bean, kestose and nystose concentrations ranged from 56–142 and 0.3–0.6 mg/100 g, respectively. Kestose levels generally increased after cooling and reheating while nystose concentration decreased **(Table 3.5)**. In cooked chickpea, kestose and nystose concentrations ranged from 24 to 41 and 1.0 to 2.2 mg/100 g, respectively. After cooling and reheating, a significant increase ($P < 0.05$) in kestose was observed in desi chickpea and a significant ($P < 0.05$) decrease in nystose was observed in kabuli chickpea **(Table 3.5)**.

Mean RS concentration ranged from 2.6–3.9 and 3.6–5.3 g/100 g in common bean and chickpea market classes after cooking, respectively **(Figure 3.1a, b)**. RS concentration generally increased after cooling and reheating for both pulse crops. Mean amylose concentration ranged from 25–33 g/100 g for common bean and 24–30 g/100 g for chickpea after cooking **(Figure 3.1c, d)**. Cooling and reheating significantly ($P <$

Table 3. 5. Fructooligosaccharide (FOS) concentrations in common bean and chickpea market classes after cooking, cooling, and reheating.

FOSs	Common bean							Chickpea	
	srb[a]	cb	gnb	lrkb	bb	nb	pb	desi	kabuli
Kestose									
Cooked	66±18a	120±56a	62±11b	142±40a	94±10b	67±26a	56±16c	24±23b	41±16a
Cooled	63±19a	119±67a	66±17b	86±14c	103±19ab	77±48a	77±30b	26±21b	50±26a
Reheated	70±26a	150±81a	96±32a	111±80b	108±33a	73±35a	103±45a	44±12a	47±25a
Nystose									
Cooked	0.3±0.3a	0.5±0.5a	0.3±0.2a	0.6±0.41a	0.6±0.2a	0.5±0.2a	0.6±0.1a	1.0±1.0a	2.2±1.7a
Cooled	0.1±0.0b	0.2±0.2b	0.1±0.1a	0.1±0.1b	0.0±0.0b	0.0±0.0b	0.1±0.1b	0.2±0.6a	0.6±0.8b
Reheated	0.1±0.1b	0.2±0.3b	0.2±0.3a	0.1±0.1b	0.1±0.2b	0.2±0.2b	0.1±0.1b	0.1±0.2a	0.4±0.4b
Total FOSs									
Cooked	66±18a	120±56a	62±11b	143±40a	94±10b	68±26a	57±16c	26±23b	43±15a
Cooled	63±19a	119±67a	67±17b	87±14c	103±19ab	77±48a	77±30b	26±20b	50±26a
Reheated	70±26a	150±81a	96±32a	112±80b	108±33a	73±35a	103±45a	44±12a	48±25a

[a]Mean (±standard deviation) values within a column followed by a different letter are significantly different at $P < 0.05$ (n = 162). Values were calculated on a wet weight basis (15% moisture). Small red bean (srb), cranberry bean (cb), great northern bean (gnb), light red kidney bean (lrkb), black bean (bb), navy bean (nb), and pinto bean (pb).

Fig 3.1

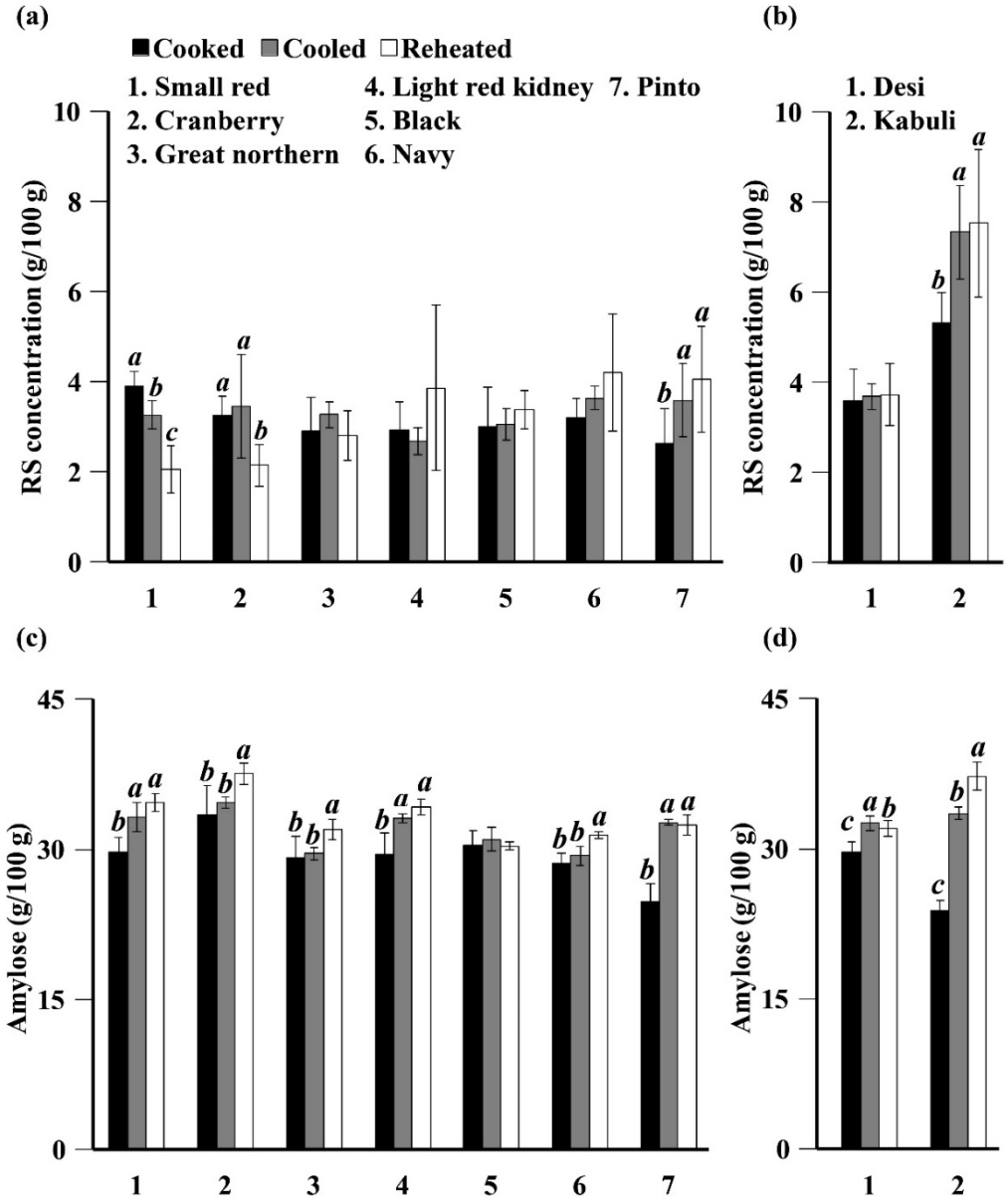

Figure 3. 1. Resistant starch (RS) and amylose concentrations of different common bean (a and c, respectively) and chickpea (b and d, respectively) market classes after cooking, cooling, and reheating. Columns and error bars represent mean values and standard deviation, respectively. Values are presented on a wet weight basis (15% moisture). Values within each market class followed by a different letter are significantly different at $P < 0.05$ (n=162).

0.05) increased amylose concentration in all pulse market classes considered except black bean **(Figure 3.1c, d)**.

SEM images of pinto bean and kabuli chickpea show starch granules that are oblong in shape. Granule size varied from approximately 17–20 μm in length and 13–17 μm in diameter for pinto bean **(Figure 3.2a)** and 17–25 μm in length and 8–17 μm in diameter for kabuli chickpea **(Figure 3.2d)**. Starch granules were relatively smaller and packed in pinto bean compared to kabuli chickpea. Swelling was observed in both cooked pulses but was more prominent in kabuli chickpea. Deformation of cell walls and starch granules were observed after cooling **(Figure 3.2b, e)** and reheating **(Figure 3.2c, f)**.

3.7. Discussion

Pulses are significant food sources of protein, minerals, vitamins, and prebiotic carbohydrates. At the global scale, common bean and chickpea provide approximately 7–13% of total daily protein, 1–3% of carbohydrates, and 1–2% of total energy requirements per day (FAO, 2019). Present study shows that a 100-g serving of cooked common bean or chickpea provides 6.8–9.7 g of prebiotic carbohydrates, i.e., 34–49% of the safe recommended intake, with the range covering the nine markets classes considered here. After cooling and reheating, total prebiotic carbohydrates declined by 3–8% in common bean but increased by 5–9% in chickpea; this shows that manipulating cooking practices can change prebiotic carbohydrate levels in pulses.

Total SA concentrations in processed pulses are a consequence of cell degradation and thermal decomposition; the former increases while the latter decreases the SA concentration. Cooking degrades the primary and secondary cell wall structures (Shomer

Fig 3.2

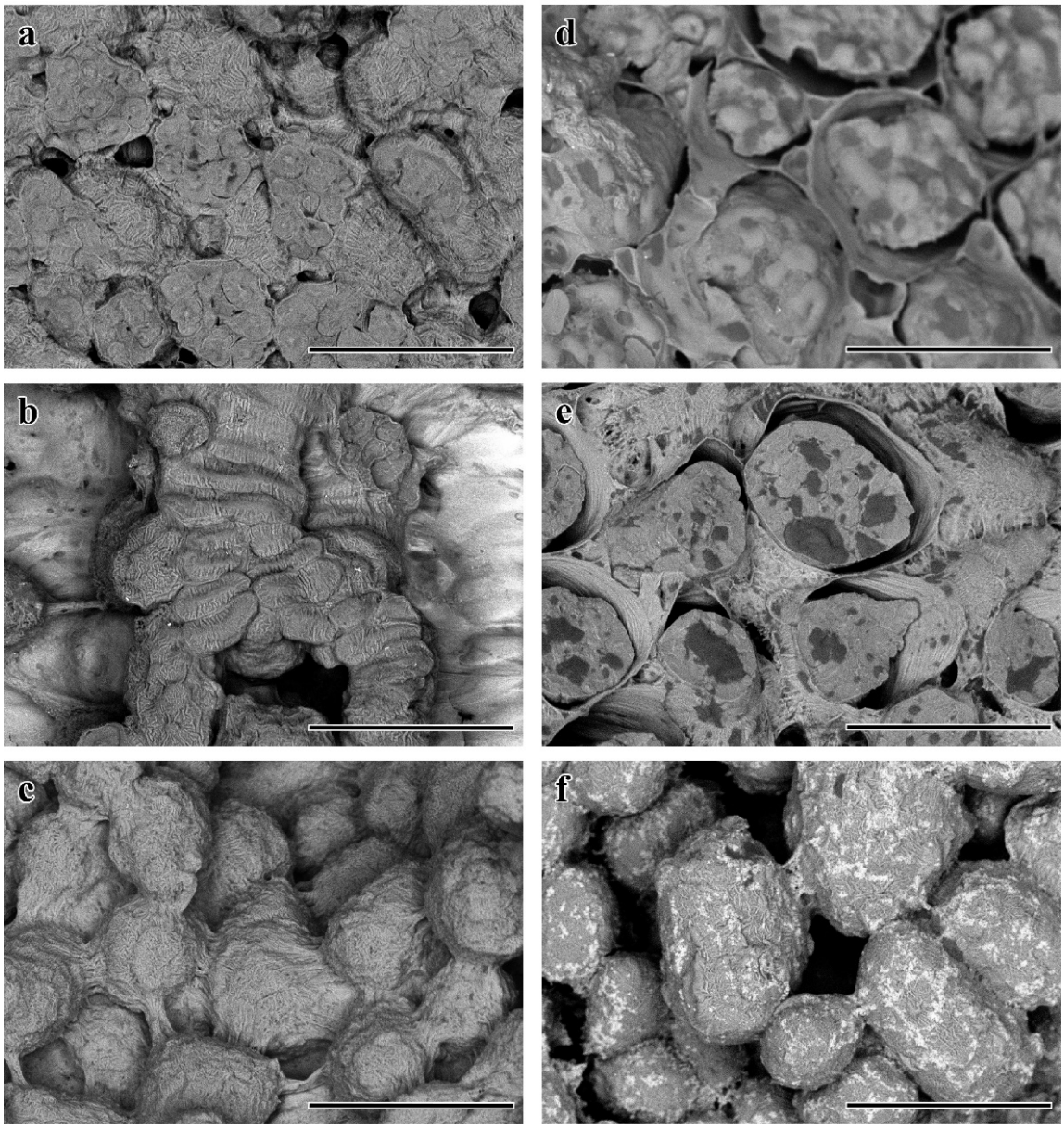

Figure 3. 2. SEM images of starch granules of (a) cooked, (b) cooled, and (c) reheated pinto bean cotyledon and (d) cooked, (e) cooled, and (f) reheated kabuli chickpea cotyledon. Scale bar for all images is 100 μm.

et al., 1990). As a result, cell wall compounds including SAs leach out into the cooking medium, increasing its concentration as observed in common bean.

In contrast, the SA levels in chickpea significantly declined after reheating, which is attributed to the higher rate of thermal degradation. Cooking at a temperature >100 °C changes the chemical structure of SAs at different moisture levels (Matsumoto et al., 2015; Matsumura, 2016). Therefore, the use of cooking temperatures below 100 °C and an intermediate moisture content medium may increase SA concentrations in pulses.

Studies conducted in pulses including the present study show that thermal treatments reduce RFO concentrations (Barampama and Simard, 1994; Bouhnik et al., 2007; Campos-Vega et al., 2018; El-Adawy, 2002). Further, reduction in RFOs is more prominent in steam-cooked pulses than pulses cooked by extrusion with low moisture (Kelkar et al., 2012). Increasing the seed:water ratio to more than 1:3 causes greater RFO reduction (Kelkar et al., 2012; Queiroz et al., 2002). During cooking, protons from water molecules act as a catalyst and cleave the glycosidic bonds in RFOs, leaving monosaccharides (galactose, fructose, and glucose) and a disaccharide (sucrose) as by-products (Forgo et al., 2013). Therefore, application of low cooking temperatures and low moisture levels may minimize RFO degradation and be used as a strategy to obtain RFO-rich pulse products.

Similar to RFOs, cooking at high temperatures decreases FOS concentrations (Courtin et al., 2009; L'homme et al., 2002). Glycosidic bonds in FOS molecules are cleaved via the proton-assisted mechanism, which is catalyzed by high moisture and acidic conditions (Courtin et al., 2009; L'homme et al., 2002). In the present study, FOS

levels increased after reheating in all cases except light red kidney bean. Tissue damage caused by re-heating may have released more cell-bound FOSs due to thermal degradation of cell tissues (Han and Baik, 2006).

Thermal treatments, i.e., cooking, cooling, and repeated heating-cooling cycles, increase the RS concentration in cereals, tubers, and pulses by 10–400% (Johnson et al., 2015; Mishra et al., 2008; Siva et al., 2018; Vasanthan and Bhatty, 1998; Yadav et al., 2009). Similarly, results of this study indicate RS levels increased by 12–54% in most common bean types and 4–42% in chickpea after cooling and reheating. At low temperatures, starch molecules (amylose and amylopectin) re-align to create more crystalline regions and, therefore, increase the RS concentration (Li et al., 2017; Perdon et al., 1999). With repeated heating and cooling, the crystalline regions and therefore RS levels are increased (Vasanthan and Bhatty, 1998). Similar to RS, amylose concentrations noted here increased after cooling and reheating, and have been shown to be positive correlated with RS (Yadav et al., 2009). Thermal energy during reheating breaks long chain amylopectin into short linear chain amylose molecules (Liu et al., 2010).

SEM images show that cooking, cooling, and reheating change the internal structure of the cell wall and starch granules **(Figure 3.2)**. When cooking in excess water, starch granules undergo an irreversible phase transition known as gelatinization, where water uptake, granular swelling, loss of double helical structure, and leaching of starch molecules can occur (Wang and Copeland, 2013). Water uptake was higher in kabuli chickpea, where more granular swelling can be seen than in pinto bean **(Table 3.1; Figure 3.2a, d)**. Deformation of starch granules during cooking and cooling shows that

amylose leaches out, generating new amylose-amylose bonds and forming RS. As noted in previous studies (Moza et al., 2012; Palav and Seetharaman, 2006; Xie et al., 2013), microwave reheating completely destroys the cell and starch granule structure **(Figure 3.2c, f)** by breaking longer starch molecules. It facilitates the re-arrangement of starch molecules to increase the crystalline regions, which leads to increasing RS levels in common bean and chickpea after reheating **(Figure 3.1)**. Present study did not provide details of cooking-induced changes in cellulose, hemicellulose, and pectin, which are also significant components of pulse carbohydrate profiles (Guillon and Champ, 2002). Therefore, further studies will be required to determine complete prebiotic carbohydrate profile changes during thermal processing before and after soaking (Fabbri and Crosby, 2016). Overall, application of a suitable temperature and seed:water ratio can increase the final prebiotic carbohydrate concentration in pulses.

3.8. Conclusion

Understanding how prebiotic carbohydrate concentrations change in different common bean and chickpea market classes during processing will help to improve the nutritional quality of pulse-based food products. This study shows that thermal treatment increases SAs and FOSs and decreases RFOs in common bean market classes. For chickpea, processing reduces SAs and RFOs and increases FOSs. Further, cooling and reheating increases RS and amylose in both pulse crops. As such, selecting appropriate pulse market classes and manipulating processing conditions can be exploited to develop prebiotic carbohydrate-rich foods.

3.9. Acknowledgments

The American Pulse Association provided funding support for this project.

3.10. References

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4. CHAPTER THREE

RETORT PROCESSING TEMPERATURE CHANGE PREBIOTIC CARBOHYDRATES CONCENTRATION IN PULSES

4.1. Hypotheses

H_0: Cooking temperature does not affect SA, RFO, FOS, RS, and amylose concentration in lentils, common bean, and chickpea market classes.

H_1: Cooking temperature affect SA, RFO, FOS, RS, and amylose concentration in lentils, common bean, and chickpea market classes.

4.2. Objective

Determine changes of SA, RFO, FOS, RS, and amylose concentration in two lentil market classes (red and green), seven common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto), and two chickpea market classes (desi and kabuli) in response to four cooking temperature ranging from 90 to 120 ºC.

4.3. Abstract

Thermal processing alters prebiotic carbohydrates concentration, yet no prior data on the impact of processing temperature in prebiotic carbohydrates in different pulse market classes. This study determined the changes in prebiotic carbohydrates [sugar alcohols (SA), raffinose family oligosaccharides (RFO), fructooligosaccharides (FOS), resistant starch (RS), and amylose] concentration in two lentil, seven common bean, and two chickpea market classes subjected to four cooking temperature ranging from 90 to

120 ºC. Seed sample of 8 g with 24 g of distilled and deionized water was sealed in a retort pouch and retorted at 90, 100, 110, and 120 ºC for 30 minutes. Samples were then analyzed for prebiotic carbohydrates using high-performance anion exchange chromatography and enzymatic assays. In lentil, SA, RFO, FOS, and amylose concentration were increased from 949 to 1181 mg/100 g, 4.7 to 6.6 g/100 g, 58 to 137 mg/100 g, and 29 to 40 g/100 g respectively with increasing processing temperature from 90 ºC to 120 ºC. In chickpea, the increase was from 1.7 to 2.4 g/100 g, 4.0 to 4.9 g/100 g, 41 to 98 mg/100 g, and 28 to 37 g/100 g. In common bean, the increase was from 18 to 26 mg/100 g, 3.3 to 6.1 g/100 g, 45 to 105 mg/100 g, and 23 to 43 g/100 g respectively. RS concentration was reduced from 1.5 to 0.5 g/100 g in lentil, 0.9 to 0.4 g/100 g in common bean, and 0.7 to 0.3 g/100 g in chickpea. Overall, total prebiotic carbohydrates concentration was increased from 7.2 to 8.4 g/100 in lentil, 4.1 to 6.7 g/100 g in common bean, and 6.5 to 7.7 g/100 g in chickpea with increasing processing temperature. This study shows that increasing processing temperature up to 120 ºC increase prebiotic carbohydrate concentration and can be incorporated into shelf-stable foods.

Key words: prebiotic carbohydrates, thermal processing, shelf-stable food, pulses

4.4. Introduction

Pulses including lentil, common bean, and chickpea are becoming more popular among consumers worldwide due its nutritional quality and related health benefits (Hall et al., 2017). Pulses are rich source of protein which provide 22–28 g of protein per 100 g of serving (USDA, 2019). Further, pulses provide 2–3 g of micronutrients and 3–17 g of low digestible carbohydrates (Biesiekierski et al., 2011; Gangola et al., 2016; Johnson et

al., 2013; Reddy et al., 1984). USA is the 7th highest pulse producer with 2.2 million tonnes of annual production (FAO, 2019). Among the total production, 60% is used for food purposes (FAO, 2019), providing 4.2 kg of annual per capita consumption in USA which is 41% less than the global pulse consumption (7.2 kg/capita/year) (FAO, 2019). Development of novel food products incorporating pulses increase pulse consumption among USA consumers and help to gain related health benefits including reduction of obesity risk.

Thermally processed shelf-stable foods, i.e. canned beans, are associated with lower risk of obesity (Luhovyy et al., 2015). Canned pulse consumption increased satiety and improve glycemic responses compared with those who consumed white bread (Wong et al., 2009). Regular consumption of canned pulses for 8 weeks reduced waist circumference (Mollard et al., 2012). Further, minimum of 3-week pulse consumption reduced low density lipoprotein and total blood cholesterol (Bazzano et al., 2011). These health benefits mainly come from low digestible carbohydrates which are often reduced by extensive thermal process (Khattab and Arntfield, 2009). Therefore, careful manipulation of processing conditions (temperature, moisture, and pressure) during pulse processing will help to retain those compounds and associated health benefits.

The impact of thermal processing including boiling, extrusion, and canning/sterilization on pulse prebiotic carbohydrates are previously reported (Berrios et al., 2010; Kelkar et al., 2012; Morales et al., 2015; Słupski and Gębczyński, 2014). Cooking pulses at 100ºC significantly reduces RFO and FOS due to thermal degradation (Courtin et al., 2009; L'homme et al., 2002). The effect of high-temperature extrusion of

pulses had inconsistent results (Berrios et al., 2010; Morales et al., 2015). Extrusion lentil at 159–161 ºC shows that raffinose level was significantly increased, verbascose was significantly decreased, and stachyose had no difference than raw lentil (Morales et al., 2015). Similar extrusion condition in another study shows that extrusion decrease raffinose and stachyose in lentil whereas increase in chickpea (Berrios et al., 2010). Further, low temperature extrusion (85 ºC) and steam cooking (82 ºC) reduced RFO in beans than its raw counterpart (Kelkar et al., 2012). Canning beans at 118–122 ºC for 16 minutes reduced RFO by 65% (Słupski and Gębczyński, 2014). Considering RS, cooking at 100 ºC did not change its concentration, but cooking with additional cooling at 4 ºC for 24 h in a refrigerator and microwaving for 1-minute increases RS by two-fold than raw seeds (Johnson et al., 2015; Siva et al., 2018). Also, sterilization of faba bean, kidney bean, and chickpea at 120 ºC for 15–20 minutes shows that RS levels were significantly reduced than its raw counterpart (Güzel and Sayar, 2012), but had more RS than ordinary boiled pulses (Güzel and Sayar, 2012).

Thermal sterilization or retort processing is a widely used technique to develop shelf-stable food products including canned pulses (Boz and Erdoğdu, 2015). The physical quality (color and texture) and the nutritional quality such as protein and micronutrients content of those retorted pulses are previously studied (Margier et al., 2018; Parmar et al., 2016). Retorted lentil, chickpea, and common bean had lower protein, dietary fiber, and magnesium concentration than the household cooked pulses (Margier et al., 2018). However, retorting also reduce anti-nutrients up to 38%, therefore, increase the bioavailability of nutrients than the household boiled pulses (Margier et al.,

2018). Even though, there are several studies measured RFO and RS concentration in retorted pulses, none evaluated the effect of retort temperature on prebiotic carbohydrate concentration in a sterilization process. Knowledge regarding this helps to develop thermally processed shelf-stable pulse food products such as canned foods with higher prebiotic carbohydrates. The objective of this study was to determine changes of SA, RFO, FOS, RS, and amylose concentration in two market classes of lentils (red and green), seven common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto) and two chickpea market classes (desi and kabuli) in response to four cooking temperature ranging from 90 to 120 ºC.

4.5. Materials and Methods

4.5.1. Materials

Chemicals were purchased from VWR International (Suwanee, GA, USA), Sigma-Aldrich (St. Louis, MO, USA), and Fisher Scientific (Asheville, NC, USA). Solvents and dilution series were prepared using distilled and deionized (ddH_2O) water resistance of ≥ 18.2 MΩ (NANO-pure Diamond, Barnstead, IA, USA).

4.5.2. Seed samples

Approximately 2 kg of commercially available lentil seeds belong to two market classes (red and green) and three processing forms (whole seed, dehulled seed, and dehulled-split seed) were obtained from the Northern Pulse Growers Association (Bismarck, ND, USA). Seven common bean market classes (small red, cranberry, great northern, light red kidney, black, navy, and pinto) and two chickpea market classes (desi and kabuli) were purchased from a commercial distributor (AGT Foods, Bismarck, ND,

USA). Samples were homogenized by hand, subsampled, and stored at room temperature (20–25 °C) until further analyses. The treatment design was a completely randomized design with five lentil types, seven common bean types, and two chickpea types (n=16), four cooking temperature (90, 100, 110, and 120 °C) (n=4), and three replicates (n=3); hence total of 192 samples.

4.5.3. Retort cooking

Approximately 8 g of sample and 24 g of ddH_2O were measured in to 12x16 cm^2 retort pouch. Then, the pouches were thermally sealed and cooked at 90, 100, 110, 120ºC for 30 minutes to in a retort (model A-142-OS, Surdry S. L., Abadiano, Bizkaia, Spain). Pouches were allowed to reach room temperature. Cooked seeds were homogenized and analyzed for prebiotic carbohydrates.

4.5.4. Determination of Moisture content

Approximately 5 g of homogenized samples were oven dried at 105ºC until it gives a constant weight (~3 hrs). Moisture content was calculated as per AACC International, 2010. Data were reported on a wet weight basis (normalized to 15% moisture).

4.5.5. Determination of SA, RFO, and FOS

A sample of 500 mg was measured and SA, RFO, and FOS were extracted as explained by Muir et al., 2009. Concentrations of those compounds were measured using high-performance anion exchange chromatography (HPAE) (Dionex, ICS-5000, Sunnyvale, CA, USA) (Feinberg et al., 2009) connected to a CarboPac PA1 column (250 × 4 mm; Dionex, CA, USA) and a CarboPac PA1 guard column (50 × 4 mm; Dionex,

CA, USA) as described by Johnson et al., 2013. Peak areas of an external reference (CDC Redberry lentil), SA (3–1000 ppm), RFO (3–1000 ppm), and FOS (3–1000 ppm) were routinely analyzed for method consistency and detector sensitivity with an error of less than 5%. The concentration of LMWCs in the samples was calculated according to Johnson et al., 2013.

4.5.6. Determination of RS

α-amylase and amyloglucosidase enzymes essay was used to measure RS concentration as per McCleary and Monaghan, 2002. The glucose concentration (C_G) resulting from enzymatic hydrolysis of resistant starch was measured using glucose oxidase-peroxidase method (GOPOD) (Megazyme, 2018). RS concentration (C_{RS}) was calculated according to $C_{RS} = (C_G \times 0.9 \times V) / m$, where m is the moisture corrected weight of sample, V is the final diluted volume, and 0.9 is a factor to convert free glucose to anhydrous glucose as occurs in starch (McCleary and Monaghan, 2002). Regular corn starch (RS concentration 1.0±0.1%, w/w) was used to check the accuracy of the data and ensured an analytical error of less than 10%.

4.5.7. Determination of amylose concentration

An enzymatic assay with α-amylase enzyme (67 U/mL) and amyloglucosidase (333 U/mL) was used to measure amylose concentration (Gibson et al., 1997). The glucose concentration resulting from enzymatic hydrolysis of amylose and total starch fractions was measured using GOPOD method (Megazyme, 2018). Absorbance was measured at 510 nm in a spectrophotometer and amylose concentration was calculated as follows:

$$\text{Amylose (\%)} = \frac{\text{Abs}_{\text{(Con A supernatant)}}}{\text{Abs}_{\text{(Total starch aliquot)}}} \times \frac{6.15}{9.2} \times 100,$$

$$\text{Amylose (g/100 g)} = \text{Total starch concentration (g/100 g)} - \frac{\text{Amylose (\%)}}{100},$$

where 6.15 and 9.2 are dilution factors.

4.5.8. Determination of total prebiotic carbohydreates

sum of SA, RFO, FOS, and RS was expressed as total prebiotic carbohydrates.

4.6. Results

In lentil, SA, RFO, FOS, and amylose were increased from 949–1181 mg/100 g, 4.7–6.6 g/100 g, 58–137 mg/100 g, and 29–40 g/100 g respectively with increasing processing temperature from 90 ºC to 120 ºC (**Table 4.1**). In chickpea, the increase was from 1.7–2.4 g/100 g, 4.0–4.9 g/100 g, 41–98 mg/100 g, and 28–37 g/100 g. In common bean, the increase was from 18–26 mg/100 g, 3.3–6.1 g/100 g, 45–105 mg/100 g, and 23–43 g/100 g respectively. RS concentration was significantly reduced ($P \leq 0.05$) from 1.5 to 0.5 g/100 g in lentil, 0.9 to 0.4 g/100 g in common bean, and 0.7 to 0.3 g/100 g in chickpea (**Table 4.1**). Overall, total prebiotic carbohydrates concentration was increased from 7.2 to 8.4 g/100 in lentil, 4.1 to 6.7 g/100 g in common bean, and 6.5 to 7.7 g/100 g in chickpea with increasing processing temperature (**Table 4.1**).

4.6.1. Sugar alcohols

Sorbitol and mannitol concentration increased from 0.8–1.1 to 1.0–1.3 g/100 g and from 26–81 to 22–83 mg/100 g in all lentil market classes respectively (**Table 4.2**). Sorbitol and mannitol concentration slightly reduced at 100 ºC and significantly increased ($P \leq 0.05$) at 120ºC than 90ºC (**Table 4.2**). In chickpea market classes, total SA significantly increased from 1.6–1.8 g/100 g to 2.3–2.6 g/100 g with increasing

Table 4. 1. Carbohydrates concentration in pulses processed at different temperatures.

Treatment	SA**,ǂ	RFO*	FOS**	RS*	Amylose*	TPC*
Lentil						
90 ºC	949±138b	4.7±0.5b	58±24bc	1.5±0.3a	29±3c	7.2±0.7b
100 ºC	851±90c	3.9±0.3c	48±35c	0.7±0.2b	35±3b	5.5±0.4b
110 ºC	1006±165b	5.0±1.0b	81±40b	0.7±0.1b	38±7ab	6.8±1.2b
120 ºC	1181±127a	6.6±1.5a	137±52a	0.5±0.1c	40±2b	8.4±1.6a
Common bean						
90 ºC	18±5c	3.3±0.3c	45±60b	0.9±0.4a	23±4d	4.1±0.5c
100 ºC	17±9c	2.6±0.5d	20±15b	0.8±0.3ab	34±4c	3.4±0.4d
110 ºC	30±8a	4.2±0.5b	81±45a	0.7±0.1b	38±6b	5.0±0.5b
120 ºC	26±6b	6.1±1.9a	105±80a	0.4±0.2c	43±3a	6.7±1.7a
Chickpea						
90 ºC	1719±137b	4.0±0.8a	41±22b	0.7±0.1a	28±6c	6.5±0.9ab
100 ºC	1539±447b	2.3±0.4b	36±20b	0.6±0.1b	31±3bc	4.4±0.8b
110 ºC	1763±201b	2.6±0.3b	43±10b	0.4±0.1c	32±1b	4.8±0.2b
120 ºC	2416±250a	4.9±2.0a	98±59a	0.3±0.0d	37±1a	7.7±2.1a

ǂMean (±standard deviation) values within a column followed by a different letter are significantly different at $P < 0.05$ (n = 168).

*g/100 g (wet basis; 15% moisture).

**mg/100 g (wet basis; 15% moisture).

SA, sugar alcohols; RFO, raffinose family oligosaccharides; FOS, fructooligosaccharides; RS, resistant starch; TPC; total prebiotic carbohydrates (sum of SA, RFO, FOS, and RS).

Table 4. 2. Prebiotic carbohydrates concentration in lentil market classes processed at different temperatures.

Lentil	Sorbitol*,ǂ	Mannitol**	SA*	Raf+Stach*	Verbascose*	RFO*	Kestose**	Nystose**	FOS**
Whole red									
90 ºC	1.1±0.1ab	26±3bc	1.1±0.1ab	3.5±0.2ab	1.0±0.1	4.5±0.3ab	45±2b	0.87±0.53a	45±2b
100 ºC	0.9±0.1b	39±2a	0.9±0.1b	2.9±0.1b	1.0±0.0	3.9±0.1b	115±10ab	0.04±0.01c	115±10ab
110 ºC	1.1±0.2ab	32±2ab	1.1±0.2ab	3.6±1.2ab	1.0±0.3	4.6±1.4ab	70±32ab	0.24±0.20bc	70±32ab
120 ºC	1.3±0.1a	22±6c	1.3±0.1a	4.5±0.9a	1.4±0.5	5.9±1.3a	153±104a	0.74±0.11ab	153±104a
Dehulled red									
90 ºC	0.8±0.0b	40±5b	0.8±0.0b	3.0±0.1b	1.6±0.1b	4.7±0.2b	77±6b	0.57±0.40	77±5b
100 ºC	0.7±0.0b	36±3b	0.7±0.0b	2.2±0.1b	1.2±0.0b	3.5±0.1b	25±4b	0.12±0.02	25±4b
110 ºC	0.8±0.2b	53±4a	0.9±0.2b	3.5±1.3ab	1.8±0.6b	5.3±1.9ab	90±75b	0.37±0.20	91±75b
120 ºC	1.1±0.1a	63±11a	1.2±0.1a	4.8±1.2a	3.0±1.0a	7.8±2.1a	184±41a	0.62±0.38	185±41a
Split red									
90 ºC	0.8±0.1ab	69±5a	0.9±0.1ab	3.2±0.1ab	1.9±0.1ab	5.1±0.2ab	72±2b	0.87±0.23a	73±2b
100 ºC	0.8±0.0b	37±2b	0.8±0.0b	2.6±0.1b	1.5±0.1b	4.1±0.1b	27±1c	0.39±0.09ab	27±1c
110 ºC	0.8±0.1b	57±8a	0.9±0.1b	3.1±0.6ab	1.7±0.3b	4.7±0.9b	69±35b	0.17±0.06b	69±35b
120 ºC	1.0±0.2a	63±10a	1.1±0.2a	4.0±0.9a	2.5±0.7a	6.6±1.6a	120±14a	0.65±0.49ab	120±14a
Whole green									
90 ºC	0.8±0.2b	71±18b	0.9±0.2b	2.7±0.6b	1.6±0.4ab	4.3±1.0ab	18±3b	0.40±0.03	19±3b
100 ºC	0.8±0.1b	45±5c	0.9±0.1b	2.3±0.1b	1.4±0.1b	3.7±0.2b	35±4b	0.63±0.14	35±4b
110 ºC	1.0±0.0ab	104±6a	1.1±0.0ab	3.2±0.0ab	1.9±0.0ab	5.1±0.1ab	95±47a	0.96±1.05	96±46a
120 ºC	1.1±0.1a	92±5a	1.2±0.1a	3.8±0.8a	2.5±0.8a	6.3±1.6a	116±11a	0.37±0.17	116±11a
Dehulled green									
90 ºC	1.0±0.1b	81±5a	1.0±0.1b	3.1±0.3bc	1.8±0.1ab	4.9±0.5b	77±6b	0.29±0.42	77±6b
100 ºC	0.9±0.0b	47±2b	1.0±0.0b	2.7±0.1c	1.5±0.1b	4.2±0.2b	38±8c	0.64±0.14	39±7c
110 ºC	1.0±0.1ab	93±6a	1.1±0.1ab	3.5±0.3ab	1.8±0.2ab	5.4±0.5ab	80±24b	0.42±0.30	80±24b
120 ºC	1.2±0.1a	83±8a	1.2±0.1a	4.1±0.6a	2.4±0.7a	6.6±1.3a	111±1a	0.21±0.30	111±2a

‡Mean (±standard deviation) values within a column followed by a different letter are significantly different at $P < 0.05$ (n = 168).
*g/100 g (wet basis; 15% moisture).
**mg/100 g (wet basis; 15% moisture).
SA, sugar alcohols (sum of sorbitol and mannitol); raf, raffinose; stach, stachyose; RFO, raffinose family oligosaccharides (sum of raffinose, stachyose, and verbascose); FOS, fructooligosaccharides (sum of kestose and nystose); RS, resistant starch; SS, soluble starch; TPC; total prebiotic carbohydrates (sum of SA, RFO, FOS, and RS).

processing temperature from 90 ºC to 120 ºC (**Table 4.3**). Overall, sorbitol and mannitol concentration were increased from 1.5–1.8 g/100 g to 2.1–2.5 g/100 g and from 88–119 mg/100 g to 97–183 mg/100 g respectively. Sorbitol and mannitol concentration were reduced at 100–110 ºC than 90 ºC and increased at 120 ºC (**Table 4.3**). In common bean, total SA was significantly increased from 14–27 mg/100 g to 22–32 mg/100 g except black bean where the concentration reduced from 20 to 17 mg/100 g (**Table 4.4**). Sorbitol concentration was significantly reduced ($P \leq 0.05$) at 100 ºC than 90 ºC and increased at 110–120 ºC. Mannitol concentration was significantly higher ($P \leq 0.05$) at 100 ºC in cranberry, great northern, and light red kidney bean (**Table 4.4**).

4.6.2. Raffinose, stachyose, verbascose and total RFO

Overall, RFO concentration in lentil was significantly increased ($P \leq 0.05$) from 4.3–5.1 g/100 g to 5.9–7.8 g/100 g regardless of the market classes when increase the processing temperature from 90 to 120 ºC (**Table 4.1**). Raffinose+stachyose, and verbascose concentration tend to reduce (2.2–2.9 and 1.01.5 g/100 g respectively) at 100 ºC than 90 ºC (2.7–3.5 and 1.0–1.9 g/100 respectively). Lentil processed at 120 ºC showed a significantly higher ($P \leq 0.05$) raffinose+stachyose (3.8–4.8 g/100 g) and verbascose concentration (2.4–3.0 g/100 g) (**Table 4.2**). In chickpea, total RFO was significantly increased ($P \leq 0.05$) in kabuli (from 3.3 g/100 g to 6.2 g/100 g) but reduced in desi (from 4.8 g/100 g to 3.6 g/100 g) (**Table 4.3**). Raffinose+Stachyose levels were increased from 2.4 to 3.2 g/100 g in desi and from 3.1 to 5.2 g/100 g in kabuli. Verbascose concentration was decreased from 2325 to 392 mg/100 g in desi and was increased from 199 to 957 mg/100 g in kabuli (**Table 4.3)**. In common bean, RFO

Table 4. 3. Prebiotic carbohydrates concentration in chickpea market classes processed at different temperatures.

Chickpea	Sorbitol*,ǂ	Mannitol**	SA*	Raf+Stach*	Verbascose**	RFO*	Kestose**	Nystose**	FOS**
Desi									
90 ºC	1.8±0.0b	88±0b	1.8±0.0b	2.4±0.0ab	2325±63a	4.8±0.1a	60±2	0.88±0.09a	61±2
100 ºC	1.8±0.2b	153±12a	1.9±0.2b	2.3±0.3b	287±22bc	2.6±0.2bc	53±4	0.63±0.09ab	54±4
110 ºC	1.9±0.0b	77±12b	1.9±0.1b	2.3±0.0b	90±16c	2.3±0.0c	42±1	0.27±0.09b	42±1
120 ºC	2.5±0.2a	97±28b	2.6±0.2a	3.2±0.7a	392±274b	3.6±1.0ab	117±83	0.47±0.45ab	117±82
Kabuli									
90 ºC	1.5±0.0b	119±9bc	1.6±0.0b	3.1±0.0b	199±11b	3.3±0.0b	21±3b	0.49±0.02	21±3b
100 ºC	1.0±0.1c	152±6ab	1.2±0.1c	1.8±0.0b	114±11b	1.9±0.0b	18±1b	0.56±0.09	18±1b
110 ºC	1.5±0.0b	117±12c	1.6±0.0b	2.7±0.1b	165±48b	2.9±0.2b	42±16b	0.43±0.26	43±15b
120 ºC	2.1±0.2a	183±28a	2.3±0.2a	5.2±0.1a	957±664a	6.2±2.1a	78±31a	0.68±0.79	79±30a

ǂMean (±standard deviation) values within a column followed by a different letter are significantly different at $P < 0.05$ (n = 168).
*g/100 g (wet basis; 15% moisture).
**mg/100 g (wet basis; 15% moisture).
SA, sugar alcohols (sum of sorbitol and mannitol); raf, raffinose; stach, stachyose; RFO, raffinose family oligosaccharides (sum of raffinose, stachyose, and verbascose); FOS, fructooligosaccharides (sum of kestose and nystose); RS, resistant starch; SS, soluble starch; TPC; total prebiotic carbohydrates (sum of SA, RFO, FOS, and RS).

Table 4. 4. Prebiotic carbohydrates concentration in common bean market classes processed at different temperatures.

Common bean	Sorbitol**	Mannitol**	SA**	Raf+Stach*	Verbascose**	RFO*	Kestose**	Nystose**	FOS**
Black bean									
90 ºC	17±2a	2±0b	20±2b	3.1±0.3bc	192±21b	3.3±0.3bc	183±18a	0.08±0.03b	183±18a
100 ºC	7±1c	2±1b	10±1d	2.1±0.1c	117±11b	2.2±0.1c	14±2c	1.18±0.11a	15±2c
110 ºC	16±1a	7±1a	23±1a	3.8±0.1b	245±35ab	4.1±0.1b	75±20b	0.15±0.19b	75±20b
120 ºC	11±0b	6±1a	17±1c	5.2±1.2a	706±472a	5.9±1.7a	128±55b	0.30±0.22b	128±55b
Cranberry bean									
90 ºC	18±1c	2±1c	20±1b	2.4±0.1c	231±9b	2.7±0.1c	14±1b	0.15±0.06b	14±1b
100 ºC	11±1d	17±1a	29±0a	3.0±0.1c	241±30b	3.2±0.1bc	28±4ab	0.57±0.21a	28±4ab
110 ºC	29±1a	4±0b	32±1a	4.0±0.4b	361±71ab	4.3±0.5b	74±26a	0.55±0.01a	75±26a
120 ºC	25±3b	4±1b	29±3a	5.1±0.9a	757±428a	5.8±1.3a	72±52a	0.68±0.28a	73±52a
Great northern bean									
90 ºC	12±1b	1±0c	14±1b	3.2±0.0bc	234±9b	3.4±0.0b	30±20b	0.12±0.00	30±20b
100 ºC	7±1c	8±0a	15±1b	2.6±0.1c	192±8b	2.8±0.1b	11±2b	0.09±0.03	11±2b
110 ºC	22±3a	6±1b	27±4a	4.4±0.7b	321±114b	4.7±0.8b	131±92ab	0.13±0.10	131±92ab
120 ºC	19±0a	6±1ab	26±1a	6.9±1.6a	1004±688a	7.9±2.3a	173±112a	0.36±0.26	174±113a
Light red kidney bean									
90 ºC	26±3b	2±3b	27±1b	3.3±0.1a	241±5	3.6±0.1ab	9±2c	0.29±0.04	9±2c
100 ºC	6±1c	25±3a	31±3ab	2.3±0.1b	345±4	2.7±0.1b	52±3b	0.31±0.32	52±3b
110 ºC	36±3a	3±0b	39±3a	3.5±0.2a	429±59	4.0±0.3a	93±27a	0.30±0.25	93±27a
120 ºC	28±7b	4±2b	32±8ab	4.1±0.8a	487±453	4.5±1.1a	10±0c	0.32±0.31	10±0c
Navy bean									
90 ºC	12±1c	1±0b	12±1c	3.0±0.1bc	201±10b	3.2±0.1bc	2±0b	0.18±0.02	2±0b
100 ºC	7±0d	7±1a	13±1c	2.0±0.1c	164±18b	2.1±0.1c	5±2b	1.08±0.05	6±2b
110 ºC	36±1a	7±0a	43±1a	4.1±0.3b	277±42b	4.3±0.3b	40±27b	0.29±0.15	40±27b
120 ºC	24±2b	6±1a	30±3b	5.4±1.3a	831±525a	6.2±1.9a	189±85a	1.57±1.89	190±85a

Common bean	Sorbitol**	Mannitol**	SA**	Raf+Stach*	Verbascose**	RFO*	Kestose**	Nystose**	FOS**
Pinto bean									
90 ºC	18±1a	2±1c	20±2b	3.4±0.0b	239±7b	3.7±0.0b	38±1b	0.44±0.13a	39±1b
100 ºC	2±1b	2±1c	4±2c	1.7±0.1c	116±10b	1.8±0.1c	11±2b	0.54±0.14a	12±2b
110 ºC	19±1a	5±1b	23±1a	3.5±0.3b	205±62b	3.7±0.3b	87±40a	0.23±0.11a	87±40a
120 ºC	18±0a	7±1a	24±1a	5.6±1.3a	799±548a	6.4±1.8a	96±16a	4.71±7.65a	101±18a
Small red bean									
90 ºC	14±0b	1±0b	14±0c	3.0±0.1c	171±26ab	3.2±0.1bc	40±9ab	0.15±0.05a	40±9ab
100 ºC	11±2c	6±1a	17±3c	2.8±0.2c	137±13b	3.0±0.2c	18±4b	0.39±0.22a	19±4b
110 ºC	18±1a	8±2a	26±2a	3.9±0.4b	207±51ab	4.1±0.4b	69±23a	0.44±0.28a	70±23a
120 ºC	15±3ab	7±0a	22±3b	5.1±0.8a	561±418a	5.7±1.2a	61±45ab	0.66±0.50a	62±45ab

ǂMean (±standard deviation) values within a column followed by a different letter are significantly different at $P < 0.05$ (n = 168).
*g/100 g (wet basis; 15% moisture).
**mg/100 g (wet basis; 15% moisture).
SA, sugar alcohols (sum of sorbitol and mannitol); raf, raffinose; stach, stachyose; RFO, raffinose family oligosaccharides (sum of raffinose, stachyose, and verbascose); FOS, fructooligosaccharides (sum of kestose and nystose); RS, resistant starch; SS, soluble starch; TPC; total prebiotic carbohydrates (sum of SA, RFO, FOS, and RS).

concentration was increased from 2.7–3.7 to 4.5–7.9 g/100 g when increase the processing temperature **(Table 4.4**). Processing at 100 ºC significantly reduced ($P \leq 0.05$) raffinose+stachyose concentration to 1.7–2.3 g/100 g in light red kidney bean and pinto bean than 90 ºC (2.4–3.4 g/100 g). However, the 110 and 120 ºC increased the raffinose+stachyose concentration in all common bean market classes. Verbascose was increased from 171–239 mg/100 g to 487–1004 mg/100 g (**Table 4.4**).

4.6.3. Kestose, nystose, and total FOS

Total FOS concentration in lentil market classes increased from 19–77 mg/100 g to 111–185 mg/100 g with increasing retort temperature from 90 ºC to 120 ºC (**Table 4.1**). Lentil kestose concentration increased from 18–77 mg/100 g to 111–184 mg/100 g (**Table 4.2**). Kestose concentration showed an increase in whole red and whole green at 100 ºC and decreased at 110 ºC. At 120 ºC, kestose concentration increased in all market classes (**Table 4.2**). Nystose concentration decreased from 0.29–0.87 mg/100 g to 0.21–0.74 mg/100 g. In chickpea, total FOS level was increased from 21–61 mg/100 g to 79–117 mg/100 g. Kestose concentration increased from 21–60 mg/100 g to 78–117 mg/100 g but only significant in kabuli (**Table 4.3**). Nystose concentration was decreased in desi while increased in kabuli. In common bean market classes, total FOS was increased in all common bean market classes except black bean when increase the processing temperature (**Table 4.4**). Kestose concentration in black bean significantly reduced from 183 to 128 mg/100 g while increased from 2–40 mg/100 g to 10–189 mg/100 g in all other market classes. Nystose concentration was increased from 0.08–0.44 mg/100 g to 0.3–1.6 mg/100 g in all common bean market classes (**Table 4.4**).

4.6.4. RS

Resistant starch concentration in studied pulses significantly reduced ($P \leq 0.05$) when increase the processing temperature from 90 to 120 ºC (**Fig 4.1**). RS concentration was reduced from 1.1–1.9 g/100 g to 0.4–0.5 g/100 g in lentil (**Fig 4.1a**), from 0.6–0.8 g/100 g to 0.2–0.3 g/100 g in chickpea (**Fig 4.1b**), and from 0.5–1.5 g/100 g to 0.3–0.37 g/100 g in common bean except light red kidney bean (**Fig 4.1c**).

4.6.5. Amylose

Amylose concentration was increased from 26–32 g/100 g to 38–43 g/100 g in lentil (**Fig 6.2a**), from 23–33 g/100 g to 37–38 g/100 g in chickpea (**Fig 4.2b**), and from 19–31 g/100 g to 37–45 g/100 g in common bean (**Fig 4.2c**) with increasing retort temperature.

4.7. Discussion

Healthy food consumption plays a major role in reducing obesity risk (Swinburn et al., 2015). However, in the modern world, people put less effort to cook themselves–a healthy way of eating–and look for ready to eat foods since it is convenient, cheap, and less time consuming (Juul and Hemmingsson, 2015; Martínez Steele et al., 2016; Moubarac et al., 2017). These ready to eat foods are often rich in fat, high in sugar, and lack in micronutrients, protein, and prebiotic carbohydrates (Steele et al., 2016). In other hand, majority of produced foods are spoiled before its consumption due to lack of post after techniques including proper storage facilities (Porat et al., 2018). Therefore, production of shelf-stable products with health benefits attain a central attention to combat obesity risk (Luhovyy et al., 2015). This study determined the prebiotic

Fig. 4.1a

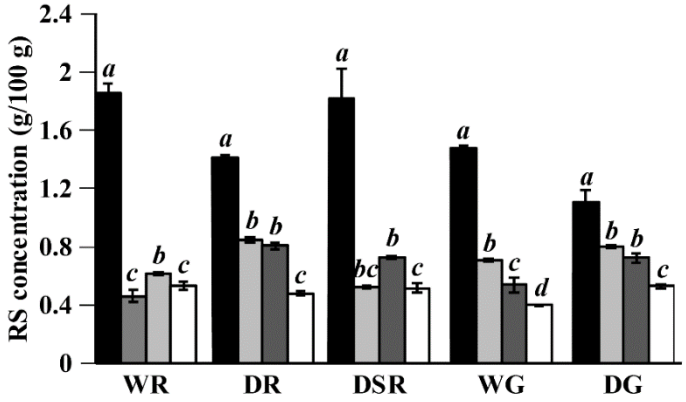

Fig. 4.1b

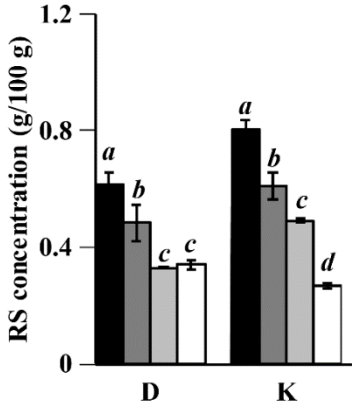

Fig. 4.1c

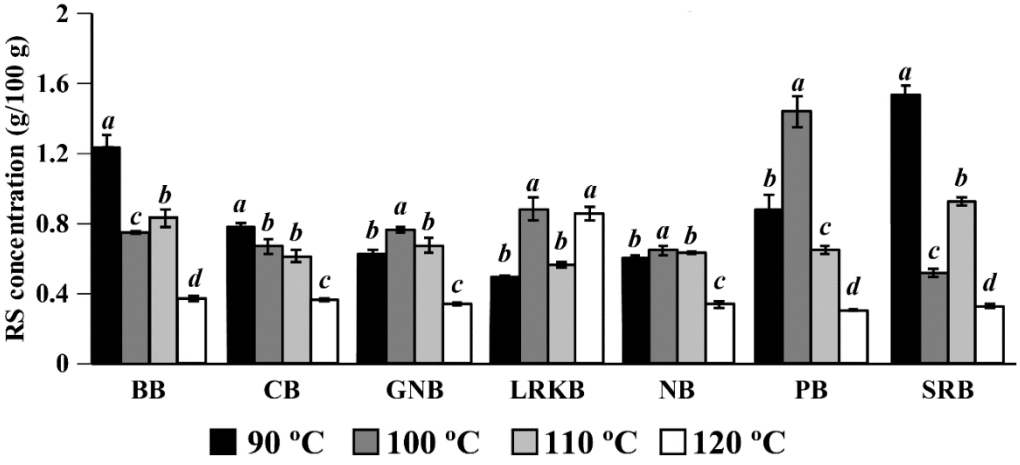

Figure 4. 1. Resistant starch (RS) concentrations of different lentil (1a), chickpea (1b), and common bean (1c) market classes cooked at 90, 100, 110, and 120 ºC. Columns and error bars represent mean values and standard deviation, respectively. Values are presented on a wet weight basis (15% moisture). Values within each market class followed by a different letter are significantly different at $P < 0.05$ (n=168). WR, whole red; DR, dehulled red; DSR, dehulled split red; WG, whole green; DG, dehulled green; D, desi; K; kabuli; BB, black bean; CB, cranberry bean; GNB, great northern bean; LRKB, light red kidney bean; NB, navy bean; PB, pinto bean; SRB, small red bean.

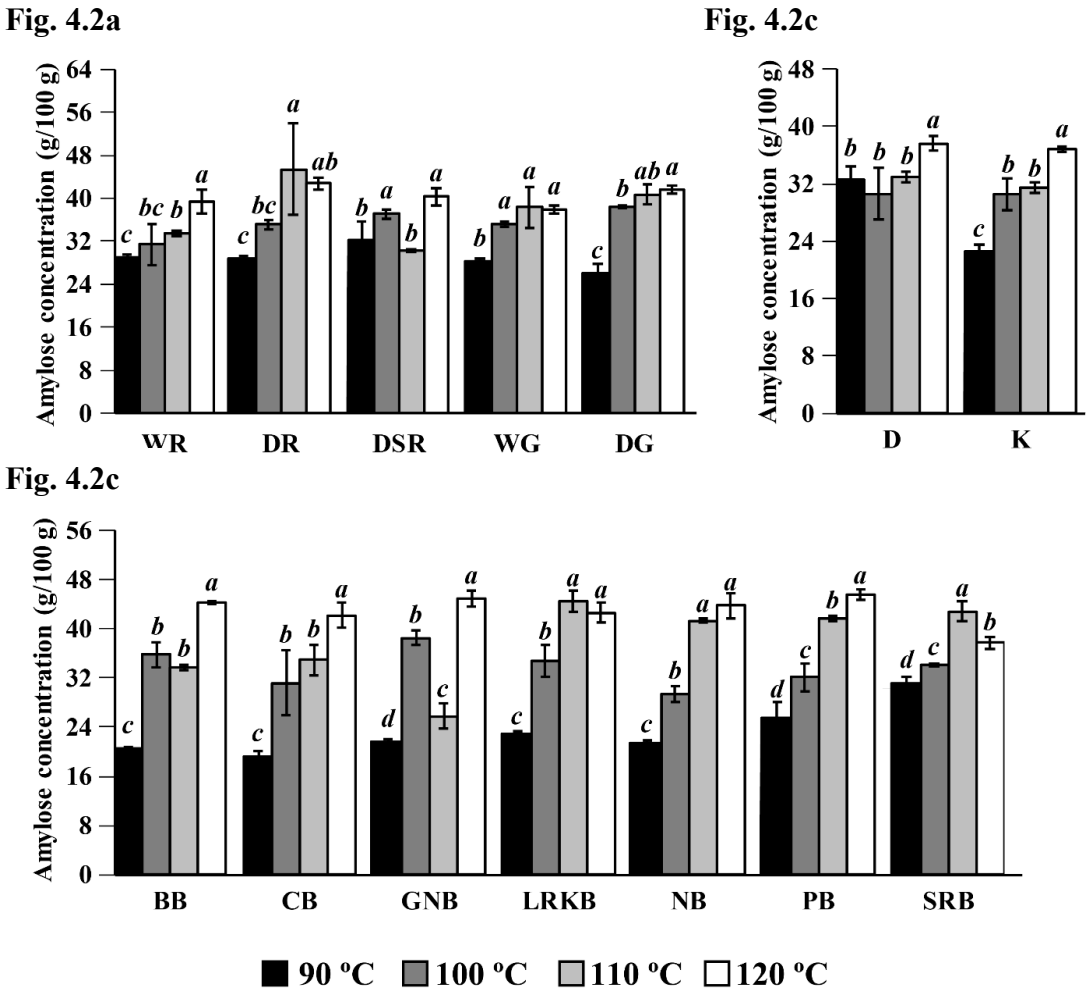

Figure 4. 2. Amylose concentrations of different lentil (2a), chickpea (2b), and common bean (2c) market classes cooked at 90, 100, 110, and 120 ºC. Columns and error bars represent mean values and standard deviation, respectively. Values are presented on a wet weight basis (15% moisture). Values within each market class followed by a different letter are significantly different at $P < 0.05$ (n=168). WR, whole red; DR, dehulled red; DSR, dehulled split red; WG, whole green; DG, dehulled green; D, desi; K; kabuli; BB, black bean; CB, cranberry bean; GNB, great northern bean; LRKB, light red kidney bean; NB, navy bean; PB, pinto bean; SRB, small red bean.

carbohydrate concentration changes with different processing temperature to get an optimum processing temperature to increase the prebiotic carbohydrates in pulses.

Total prebiotic carbohydrates concentration increased by 17, 63, 18% in lentil, chickpea, and common bean market classes when increase the retort temperature from 90ºC to 120ºC. Increasing temperature can reduce the processing time (Boz and Erdoğdu, 2015) and therefore reduce the thermal degradation of protein and micronutrients (Chitra et al., 1996). Therefore, shelf-stable pulses that is processed at high temperature for a short time is an ideal food for modern consumers to get food related health benefits.

High temperature processing causes thermal decomposition of low molecular weight carbohydrates (LMWC) in front of excess water (Courtin et al., 2009; Forgo et al., 2013; L'homme et al., 2002; Matsumoto et al., 2015; Matsumura, 2016). Structural changes in SA (Matsumoto et al., 2015; Matsumura, 2016) and breaking of glycosidic bonds in RFO and FOS cause the thermal degradation (Courtin et al., 2009; L'homme et al., 2002). However, thermal processing also degrades the primary and secondary cell wall structures as well as disrupt the cells (Shomer et al., 1990). Consequently, LMWC leach out to the medium, which increase those concentrations. Therefore, the concentration of LMWC in thermally processed foods is the net result of thermal decomposition of LMWC and cell degradation of seeds (**Fig 4.3**).

Concentration of LMWC showed a decreasing trend in pulses processed at 100–110 ºC and increased at 120 ºC than pulses processed at 90 ºC. Net concentration of those compounds may be the result of (1) thermal decomposition; decrease the LMWC concentration and (2) cell degradation; increase the LMWC concentration (**Fig 4.3**). At 90–110 ºC, thermal decomposition may higher than the cell degradation, which decreases overall LMWC concentration. At 120 ºC,

the cell degradation may be higher than the thermal decomposition that increases overall LMWC concentration (**Fig 4.3**).

Fig. 4.3

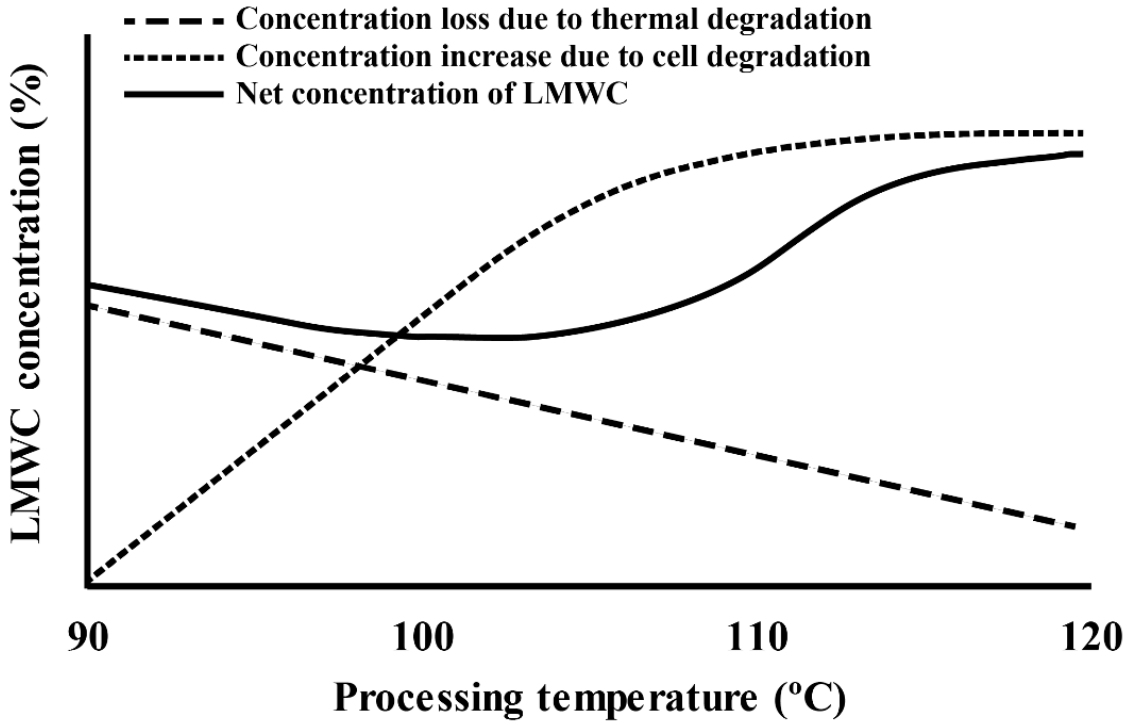

Figure 4. 3. Proposed mechanism of increased concentration of low molecular weight carbohydrates (LMWC) in pulses with increased processing temperature (90–120 ºC). Net concentration of LMWC in whole pulse seeds depends on the thermal degradation of LMWC (which decrease the LMWC concentration) during thermal process and cell degradation which release the trapped LMWC to the medium.

Present study shows that increasing processing temperature reduced RS concentration. Similar results were observed in Indian lentil where traditional cooking at 90–100 ºC has slightly higher RS than lentil cooked at 121 ºC (Mahadevamma and Tharanathan, 2004). Also, canning common beans at 116 ºC for 42 minutes reduce RS content by more than 54% (Pedrosa et al.,

2015). However, processing high amylose corn starch at 140–145ºC increased RS content by 25–30% (Dundar and Gocmen, 2013). Also, autoclaved banana starch showed a higher RS content than its raw counterpart (González-Soto et al., 2004). These different observations among pulse, corn, and banana starch may be due to differences of starch granule's physical characteristics (i.e. starch granule size and relative crystallinity) and chemical composition (i.e. amylose: amylopectin ratio)—major factors for the formation of RS (Bajaj et al., 2018).

RS concentration has positive correlation with amylose concentration (Yadav et al., 2009) since mainly amylose molecules are responsible for the formation of RS. However, in this study, RS concentration was decreased in pulses with increasing temperature while amylose concentration was increased. Cooking pulses in a water medium allows starch granules of pulse seeds to absorb water (Ai and Jane, 2015; Carlstedt et al., 2015). Beyond certain point, the starch granules are rupture and allow amylose and amylopectin molecules to leach out to the cooking medium increasing those concentrations (Ai and Jane, 2015; Carlstedt et al., 2015). Released amylose and amylopectin re-align themselves making crystalline regions depending on those chain lengths and form RS (Wang et al., 2015). However, high temperature process such as retort processing at high pressure may fragment amylose and amylopectin molecules and make short chain molecules, which do not have the capability to make crystalline regions to form RS (Eerlingen and Delcour, 1995).

The present study did not measure the impact of storage time and storage temperature on formation of RS which are two important parameters to increase the RS content in thermally processed foods (González-Soto et al., 2007; Namratha et al., 2002). We did not measured changes in the other high molecular weight prebiotic carbohydrates such as cellulose, hemicellulose, and pectin with increasing temperature. Further studies will be required to

measure the concentration changes of those compounds and to determine the impact of seed: water ratio and pressure effect on prebiotic carbohydrates in pulses. Overall, retorted lentil, chickpea, and common bean can provide 28–42, 22–39, and 17–34% of recommended safe daily intake of prebiotic carbohydrates (recommended safe daily intake of prebiotic carbohydrates assumed as 20 g/day as per (Douglas and Sanders, 2008).

4.8. Conclusion

Understanding the impact of processing temperature on prebiotic carbohydrates in pulses help to develop shelf-stable pulse products rich in prebiotics. Increasing retort temperature from 90 ºC to 120 ºC increased concentration of SA, RFO, and FOS, and amylose, but decreased RS level. Overall, our study shows that 100 g of retorted pulses can provide 3.4–8.4 g of prebiotic carbohydrates, which is 17–42% of recommended safe daily intake.

4.9. Acknowledgements

Funding support for this project was provided by the USDA National Institute of Food and Agriculture [Plant Health and Production and Plant Products: Plant Breeding for Agricultural Production program area (grant no. 2018-67014-27621/project accession no. 1015284)] and the American Pulse Association.

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5. GENERAL DISCUSSION

Lentil, common bean, and chickpea belong to pulse crops and had low calorie values due to low fat and high levels of low digestible carbohydrates including SA, RFO, FOS, RS, cellulose, hemicellulose (de Almeida Costa et al., 2006; Johnson et al., 2013). These prebiotic carbohydrate concentrations are changed during food processing (Abdel-Gawad, 1993; Han and Baik, 2006; Johnson et al., 2015; Siva et al., 2018; Verde et al., 1992). Therefore, the information about the prebiotic carbohydrate profiles of pulse market classes and the effect of cooking, cooling, and reheating on those prebiotic carbohydrates are important. Further, effect of cooking temperature on pulse prebiotic carbohydrates is important to get optimum concentration of prebiotic carbohydrates in pulses to produce thermally processed shelf-stable pulse foods.

Prebiotic carbohydrate profiles change among pulses market classes. The fisrt study shows that out of 51-54 g/100 g of total carbohydrates, lentil, common bean, and chickpea provides 12, 15, and 12 g of prebiotic carbohydrates. In lentil market classes, red lentil, specifically red split lentil (11–13 g/100 g) had higher prebiotic carbohydrates than green lentil (10–11 g/100 g). In common bean, navy bean had higher prebiotic carbohydrates (16 g/100 g) than small red, cranberry, great northern, light red kidney, black, and pinto bean (14–15 g/100 g). In chickpea, kabuli had higher prebiotic carbohydrates (13 g/100 g) than desi (11 g/100 g). These observations highlight that plant selection and breeding of relevant pulse market classes can increase the prebiotic carbohydrate levels in pulses and help to select relevant pulses to incorporate into diets to increase the prebiotic carbohydrates intake.

Manipulation of food processing method can be used to increase the nutritional value of pulses (Abdel-Gawad, 1993; Han and Baik, 2006; Johnson et al., 2015; Siva et al., 2018; Verde et al., 1992). The second study shows that 100 g of cooked common bean and chickpea provide

7–8 g and 8–10 g of prebiotic carbohydrates respectively. Cooling and reheating had different effect on pulse market classes. Cooling did not change prebiotic carbohydrate concentration in common bean, but significantly increased in chickpea market classes. Also, reheating significantly decreased prebiotic carbohydrates in common bean and did not change in chickpea. Understanding the mechanisms of these changes and associated physical and chemical factors are important to study for optimize the processing condition to each pulse market classes to increase the prebiotic carbohydrates in processed pulses.

Consumption of thermally processed shelf-stable foods, i.e. canned beans, are becoming more popular due to their low cost and convenience (Juul and Hemmingsson, 2015). Nutritional quality of those foods should be considered due to increased risk of obesity upon unhealthy food consumption (St-Onge et al., 2003). The third study evaluated the impact of retort processing temperature on prebiotic carbohydrates of pulses. Total prebiotic carbohydrates concentration was increased from 7 to 8 g/100 in lentil, 4 to 7 g/100 g in common bean, and 7 to 8 g/100 g in chickpea when increase the processing temperature from 90 ºC to 120 ºC. This study shows that increasing processing temperature up to 120 ºC increase prebiotic carbohydrate concentration. However, the impact of moisture and processing pressure on prebiotic carbohydrates should be further investigated.

Food based intervention is one of the effective approaches to reduce childhood obesity (Sallis and Glanz, 2009). Food based interventions are interconnected to agriculture, industrial food producers, and policy makers (Story et al., 2009; Verduin et al., 2005). Agriculture based Universities and research centers should breed crops including pulses that are nutritionally superior and adapted to harsh environmental conditions. On other hand, food industry should provide healthy food options at low cost via optimizing the processing conditions (Verduin et al.,

2005). Development of processed foods using healthy ingredients including pulses rich in prebiotic carbohydrates may be a successful food-based approach to increase healthy food consumption.

6. CONCLUSIONS AND FUTURE DIRECTION

Lentil, common bean, and chickpea are rich sources of prebiotic carbohydrates including SAs, RFOs, FOSs, RS, hemicellulose, and cellulose, which can be changed during cooking, cooling, and reheating. Increasing cooking temperature from 90 ºC to 120 ºC increased prebiotic carbohydrates in pulses by17–63%. However, the impact of moisture and processing pressure on prebiotic carbohydrates during processing need to be studied. Also, the effect of processed pulse market classes on animal/human obesity biomarkers are yet be evaluated. Therefore, the future pulse research will include,

1. Determination of impact of moisture and pressure on lentil, common bean, and chickpea market classes in response to thermal processing.
2. Precooked shelf-stable pulse diet on obesity biomarkers and gut microbiome using animal/human subjects.
3. Development of pulse-based shelf-stable food products such as pulse spreads, morning cereals, and pasta to provide optimum prebiotic carbohydrates to children.

As a conclusion, pulse can be incorporated into shelf-stable foods as a whole food or as an ingredient to increase the nutrient quality of a diet.

6.1. References

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